Effect of stone and roller milling on physicochemical, functional and structural properties of sifted wheat flour

by

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Abstract

Stone milling, which is one of the oldest forms of wheat flour milling, makes use of two stones to grind wheat grain to a flour. Stone milling is often associated with whole wheat flour, however a range of stone milled flour, including white flour, is found in supermarkets and other retail points across South Africa. Very few studies have focussed on white stone milled flour, and this study set out to provide fundamental knowledge regarding this.

The first research chapter studied the effect of stone and roller milling on the physicochemical, functional and structural properties of white flour. Two hard wheat grain samples, i.e. a South African commercial wheat cultivar and an imported Canadian commercial blend, were milled using stone and roller milling methods. The results obtained from the samples indicated that the milling methods used often had the largest effect on the flour samples, larger than the wheat types or the interaction of wheat type and milling method. Sieving the stone ground wheat meal through a 212 µm sieve (as per South African wheat flour regulations) proved ineffective in removing the bran particles, as indicated by the significantly darker colour and significantly higher ash content compared to the roller milled flour. The sifted stone milled flour had an extremely low flour yield compared to the roller milled flour. This may be because the stone mill crushes the entire wheat kernel to produce a whole wheat flour and does not separate the bran and endosperm as is the case with the break system of a roller mill. The ash content of the sifted stone milled flour was significantly higher than that of the refined roller milled flour. The stone milled flour also had a significantly higher water absorption capacity than the roller milled flour, which was due to the significantly smaller median flour particle size, higher starch damage and ash content. The higher ash content suggests a higher aleurone and bran content, thus a higher arabinoxylan content and water absorption capacity. Stone milled samples had significantly higher falling numbers than roller milled samples. Significant differences between stone and roller milled flour for both wheat samples were seen for the alveograph P (tenacity), L (extensibility), P/L (curve configuration ratio) and W (deformation energy). The tenacity and curve configuration ratio were significantly higher, and the extensibility and deformation significantly lower for stone milled flour compared to the roller milled flour. The mixograph midline peak time and peak height were mostly affected by the wheat types, however the stone milled flour samples had significantly lower values for both these aspects compared to the roller milled samples. The pasting properties obtained with the Rapid Visco Analyser (RVA) also significantly differed between milling methods: the stone milled flour had lower pasting viscosities and peak time, but a higher pasting temperature. The scanning electron micrographs (SEM) provided insight into the qualitative aspects of

the flour samples and illustrated that stone milled flour samples were less uniform with visible mechanical damage to the starch granules.

The second research chapter aimed to determine the adherence of three commercial stone milled flour samples and one roller milled sample to South African wheat flour and fortification regulations. The samples were sourced from points of retail in the Western Cape, South Africa, and the moisture content, ash content, bran content, crude protein content, CIELab colour and presence of iron were determined. The protein content suggests that the flour samples could be suitable for bread production, yet the bran content of the stone milled flour samples were too high to be classified as white bread wheat flour. The packaging of the roller milled flour samples indicated that the products were fortified with micronutrients, yet one batch did not indicate a presence of iron. None of the stone milled samples indicated a presence of iron, nor did the packaging contain fortification claims or information as stipulated in the regulations.

Uittreksel

Die metode om koring met klippe te maal is een van die oudste vorms van koring maal. Twee klippe word teen mekaar geskuur om die koring tot ‘n meel, oftewel steengemaalde meel, te maal. Steengemaalde meel word dikwels geassosieer met volgraanmeel, alhoewel ‘n wye reeks steengemaalde meel, insluitend witmeel, in supermarkte en ander verkoopspunte in Suid-Afrika verkoop word. Die doel van hierdie studie is om fundamentele kennis rakende wit steengemaalde meel in te samel omdat min vorige studies daarop fokus.

Die eerste navorsingshoofstuk bestudeer die effek van steen- en rollermaalmetodes op die fisikochemiese, funksionele en strukturele eienskappe van witmeel. Twee koringmonsters, naamlik ‘n Suid-Afrikaanse kommersiële koringkultivar en ‘n ingevoerde Kanadese kommersiële mengsel, is gemaal met steen- en rollermetodes. Die resultate dui aan dat die maalmetodes die grootste effek op die monsters gehad het in vergelyking met die effek van die koringtipes of die kombinasie van koringtipe x maalmetode. Die gesifde steengemaalde meel het ‘n laer opbrengs gelewer teenoor die rollermeel. Die wit steengemaalde meel is geproduseer deur die volgraanmeel deur ‘n 212 µm-sif (soos per Suid-Afrikaanse meel regulasies) te sif, maar dit was oneffektief in die verwydering van die semel. Die hoë semelinhoud is aangedui deur die betekenisvolle verskil in kleur- en asinhoud. Die rede vir die hoë semelinhoud is as gevolg van die steenmeul wat die hele koringkorrel maal om volgraanmeel te produseer. Dit verskil van ‘n rollermeul omdat dié ‘n stap het wat die semel en endosperm skei voor die endosperm verklein word tot meel. Die asinhoud van die steengemaalde meel (van beide koringmonsters) was te hoog om as witbroodkoringmeel, volgens Suid-Afrikaanse regulasies, geklassifiseer te word. Die steengemaalde meel het ook ‘n hoër betekenisvolle waterabsorpsiekapasiteit gehad as die rollermeelmeul as gevolg van die betekenisvolle hoër styselbeskadiging en asinhoud, asook die betekenisvolle kleiner mediaan meelpartikelgrootte. Betekenisvolle verskille tussen die steen- en rollermele se alveograaf P (weerstand van die deeg tot verlenging), L (rekbaarheid), P/L (kurwekonfigurasieverhouding) en W (deformasie-energie) is waargeneem. Die steengemaalde meel se P en P/L was betekenisvol hoër, en die L en W betekenisvol laer as dié van die rollermeel. Die verdikkingseienskappe van die *Rapid Visco Analyser* (RVA) het ook betekenisvol verskil tussen maalmetodes: die steengemaalde meel het laer verdikkingsviskositeite en piektye gehad, asook ‘n hoër verdikkingstemperatuur. Die skanderende elektronmikrograwe (SEM) het die kwalitatiewe aspekte van die meelmonsters aangedui, asook die oneenvormigheid van die steengemaalde meelmonsters en die meganiese skade wat die stysel ondergaan het.

Die tweede navorsingshoofstuk se doel was om die nakoming van drie kommersiële steengemaalde meel- en een rollermeelmonster tot Suid-Afrikaanse koringmeel- en fortifiseringsregulasies te bepaal. Die monsters is afkomstig van supermarkte en ander verkoopspunte in die Wes-Kaap, Suid-Afrika. Die vog-, as-, semel- en proteïeninhoud, asook die CieLab-kleur en ysterteenwoordigheid is bepaal. Die proteïeninhoud dui aan dat die meelmonsters geskik is vir broodproduksie, alhoewel die asinhoud te hoog was om die steengemaalde meel te klassifiseer as ʻn witbroodmeel. Die verpakking van die rollermeelmonsters dui aan dat die produkte gefortifiseer is met mikronutriënte, alhoewel een monster nie ʻn ysterteenwoordigheid aangedui het nie. Geen van die steengemaalde monsters het ʻn teenwoordigheid van yster aangedui nie.

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List of Abbreviations

%	Percentage
°C	Degrees Celsius
a*	CIELab red-green value
AACC	American Association of Cereal Chemistry
ANOVA	Analysis of variance
b*	CIELab blue-yellow value
BR	Blend wheat roller milled flour
BS	Blend wheat stone milled flour
BSE	Back Scattered Electron
Ca.	Circa
cm	centimetre
cP	Centipoise
CR	Cultivar wheat roller milled flour
CS	Cultivar wheat stone milled flour
D50	Median particle size
dH_2O	Distilled water
e.g.	*exempli gratia* (for example)
Eq.	Equation
et al.	*et alibi* (and elsewhere)
FBS	Fixed bottom stone
FE-SEM/FESEM	Field Emission Scanning Electron Microscope
FN	Falling number
FTS	Fixed top stone
g	Grams
GI	Gluten Index
h	Hour
HI	Hardness Index (Single Kernel Characterisation System)
i.e.	*id est* (that is)
J	Joules
kg	Kilograms
kV	Kilovolt
kW	Kilowatt
L	Alveograph extensibility
L*	CIELab lightness value
LSD	Least significant difference
m	Meter
mb	Moisture basis
mg	Milligram
min	Minutes

mL	Millilitre
mm	Millimetre
N	Nitrogen
n	Number of samples
nA	Nano Ampere
NaCl	Sodium chloride
NIR	Near Infrared Reflectance
P	Alveograph tenacity
P/L	Alveograph curve configuration ratio
PC	Principal component
PCA	Principal component analysis
RM	Roller mill
rpm	Rotations per minute
RVA	Rapid Visco Analyser
s	Seconds
SAGL	South African Grain Laboratory
SD	Standard deviation
SEM	Scanning Electron Microscope
SKCS	Single Kernel Characterisation System
SM	Stone mill
TFC	Total flavonoid content
Vb	Breakdown viscosity
Vf	Final viscosity
Vp	Peak viscosity
Vs	Setback viscosity
vs.	Versus
Vt	Trough viscosity
W	Alveograph deformation energy
WAC	Water absorption capacity
µm	Micrometre

The language, style and referencing format used are in accordance with the requirements of the *International Journal of Food Science and Technology*. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

CHAPTER 1
Introduction

An essential role player in global food security, wheat is considered one of the 'big three' crops, the other being maize and rice (Shewry & Hey, 2015; Shiferaw *et al*., 2013). Wheat, which is seen primarily as a carbohydrate, is beneficial to human health because it also contains proteins, fibre, vitamins, minerals, lipids and phytochemicals (Shewry & Hey, 2015). Wheat is milled to produce flour which is used in a range of products such as biscuits, pasta and bread. Wheat flour has unique dough forming capabilities which are essential for bread production (Wrigley, 2016). The development of a gluten network upon addition of water and mixing allows the dough to hold starch granules and gas cells. Roller milling is the most predominant method in the milling industry, however stone milling is also prevalent, especially small-scale stone mills (Doblado-Maldonado *et al.*, 2012; Ross & Kongraksawech, 2018). Commercial stone millers often combine stone and roller milling methods to ensure the quality and yield of the flour are acceptable. This allows the product to still be called a 'stone milled' flour (Doblado-Maldonado *et al.*, 2012). However, as there is no previous published work on this topic (to the author's knowledge), combination milling needs to be studied further.

The roller milling process consists of various systems: the break, sizing, reduction and tailings systems (Delcour & Hoseney, 2010; Posner & Hibbs, 2011). Metal rollers rotate in opposite directions and are precisely gapped to allow the wheat kernel to be sheared open for endosperm retrieval from the bran and germ. The endosperm particles are then gradually reduced in size to form a white flour. Depending on the milling stage, the rollers are either corrugated (as in the break system) or smooth (as in the reduction system). Sieving between steps ensures the larger particles (which mostly consist of bran) to be separated from the middlings (or large endosperm particles) (Delcour & Hoseney, 2010). The endosperm particles are then reduced in size in order to pass through a 212 µm sieve in order to produce a flour in adherance to South African wheat flour regulations (Department of Agriculture, Forestry and Fisheries, 2017).

Stone mills are usually small-scale, regional mills that mill local, organic or ancient wheats to produce wheat flour (Cappelli *et al*., 2020a; Kihlberg *et al*., 2004; Ross & Kongraksawech, 2018). Stone milled flour is often perceived as a niche, artisanal product that is more nutritional and flavoursome than roller milled flour, resulting in a marketing advantage associated with labelling a product 'stoneground' or 'stone milled' flour (Albergamo *et al*., 2018; Cubadda *et al*., 2009; Guerrini *et al*., 2019; Ross & Kongraksawech, 2018; Di Silvestro *et al*., 2014). This may be due to stone milled flour traditionally being whole wheat flour, which is rich in fibers, vitamins, minerals and other micronutrients. Stone milling entails grinding a single grain stream between two stones using shear, compression and abrasion forces to produce a whole wheat flour with a 100% extraction rate (Doblado-Maldonado *et al.*, 2012; Gèlinas *et al*., 2004). To achieve a refined or semi-refined flour, the whole wheat flour is passed through a set of centrifugal sieves or plansifters to remove any larger flour or bran particles (Cappelli *et al*., 2020b). Due to the entire wheat kernel being crushed to the same particle size during stone milling, separating the bran from the endosperm by sieving is often ineffective as the bran is distributed throughout the flour (Gèlinas *et al*., 2004). This problem is further exacerbated by larger endosperm particles remaining attached to the bran particles.

Most studies focus on whole wheat stone milled flour, and there has been only a very small interest in refined white stone milled flour (Cappelli *et al*., 2020a; Palpacelli *et al*., 2007). Scarcely any previous work evaluates the physicochemical and functional properties of white stone milled flour, such as the alveograph, mixograph and pasting properties. Scanning electron microscopy could also provide qualitative information regarding the structural differences between stone and roller milled flour, however this has not been done in previous studies. The designs and setting of stone mills settings vary, and the subjective process is usually reliant on the stone millers and their expertise (Ross & Kongraksawech, 2018). Stone mills are often associated with higher operational temperatures than roller mills, with heat being generated by the friction of the stones and the grain (Posner & Hibbs, 2011). The high temperature, grinding severity and long dwelling time (or milling duration) may affect the properties of the stone milled flour (Prabhasankar & Rao, 2001).

Whole wheat stone milled flour is typically characterised with having high levels of starch damage and water absorption (Prabhasankar & Rao, 2001). Contrastingly, Kihlberg *et al*. (2004) indicated that the starch damage of stone milled flour was less than that of roller milled flour. The particle size distribution of stone milled flour also varied between studies as the milling duration and mill adjustments vary. Stone milled flour samples can be coarser than roller milled samples (Gélinas *et al*., 2004; Palpacelli *et al*., 2007), or finer (Ross & Kongraksawech, 2018). The particle size distribution, along with the bran content, affects the flour's water absorption, pasting, gluten and mixing properties.

White stone milled flour and a variety of products containing stone milled flour (such as bread, rusks, biscuits and tortilla wraps) are found in supermarkets and other retailers across South Africa. 'Stone milled' or 'stone ground' flour is not a defined term in South African wheat flour regulations. However, 'white bread wheat flour' (or 'white bread flour made from wheat') is a defined wheat class in regulations, and stone milled versions of this are available. To be classified as white bread wheat flour according to

South African wheat flour regulations, the ash content of the flour must be between 0.60% and 1.0% and must contain no separated wheat bran, germ or semolina (Department of Agriculture, Forestry and Fisheries, 2017). Wheat flour must also be fine enough to pass through a 212 µm wire mesh sieve. No previous work has focussed on commercially available stone milled flour, especially not in South Africa where it is becoming increasingly easy to find in supermarkets and retailers.

The first part of this study aimed to investigate the physicochemical, functional and structural properties of white stone milled flour and to compare it to white roller milled flour produced from the same wheat samples. The aim of the second part of this study was to evaluate three commercially available white stone milled flours and one roller milled flour according to South African wheat flour regulations (Department of Agriculture, Forestry and Fisheries, 2017; Department of Health, 2016). This includes the classification of the flour samples and the analysis of several quality aspects, such as the ash, bran, moisture, protein and colour, as well as the presence of iron to indicate if fortification took place.

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CHAPTER 2
Stone milling of wheat: a critical review

2.1 Introduction

Wheat has been ground to flour using stones for thousands of years. The design of the modern stone mill has since developed from two stones being moved against each other by hand into an efficient apparatus that can be used to produce either whole wheat or a refined white flour. Today, stone milling is associated with organic and ancient wheats that are produced by small-scale and regional mills, with limited fundamental research and legislation available (Gélinas *et al*., 2004; Guerrini *et al*., 2019; Kihlberg et al., 2004; Ross & Kongraksawech, 2018). Most studies are based on whole wheat flour, as traditionally stone milled flour was produced by crushing the entire wheat kernel between two stones to produce a whole wheat flour. Recently, interest in white stone milled flour has emerged as this product is found to be available commercially (Cappelli *et al*., 2020a). Stone millers often make use of a combination of stone and roller milling to achieve a superior quality stone milled flour, yet still maintain the marketing advantage of using the term 'stone milled' or 'stoneground' (Doblado-Maldonado *et al*., 2012).

Previous studies on stone milling have indicated that the settings of the stone mill and the milling process influenced the temperature and mechanical damage of the flour, which in turn influenced the physicochemical composition and eventually the sensory characteristics of the bread loaf. Research regarding stone milling presented contrasting results regarding flour properties such as particle size distribution, starch damage, water absorption and heat generated during milling, possibly due to the different mills and their respective settings such as feed rate, aperture, rotational speed, abrasiveness and wheat tempering (Cappelli *et al*., 2020b; Di Silvestro *et al*., 2014; Gélinas *et al*., 2004; Kihlberg *et al*., 2004; Prabhasankar & Rao, 2001; Ross & Kongraksawech, 2018). Limited work is available on the sensory aspects of stone milled flour products such as bread and chapatti (Ghodke *et al*., 2009; Kihlberg *et al*., 2004).

Stone milled flour is often perceived as more nutritious, and studies have indicated that whole wheat stone milled flour has a less severe loss of micronutrients and elements (Albergamo *et al*., 2018; Ficco *et al.*, 2016), as well as a larger decrease in mycotoxins such as vomitoxin and zearalenone (Palpacelli *et al.*, 2007) than white roller milled flour.

This review aims to provide an overview of the knowledge available on stone milled wheat flour. This entails a history of milling, as well as the effect of the stone mill's settings on the physicochemical, functional and nutritional properties of flour.

2.2 A brief history of milling

Charred bread-like remains and preserved wild einkorn wheat (*Triticum boeticum/urartu*), barley (*Hordeum spontaneum*) and oats (*Avena* sp.) were found on a Natufian hunter-gatherer site in what is known today as Jordan, dating back to 14 400 years ago (Arranz-Otaegui *et al.*, 2018). The scanning electron micrographs of these bread-like products clearly showed pericarp, bran, endosperm and starch particles having broken edges, which indicate that the wheat was grinded. It is probable that the method of decreasing the particle size of these wild cereals was by crushing it between two rocks. A timeline of the development of the wheat mill is illustrated in Figure 2.1.

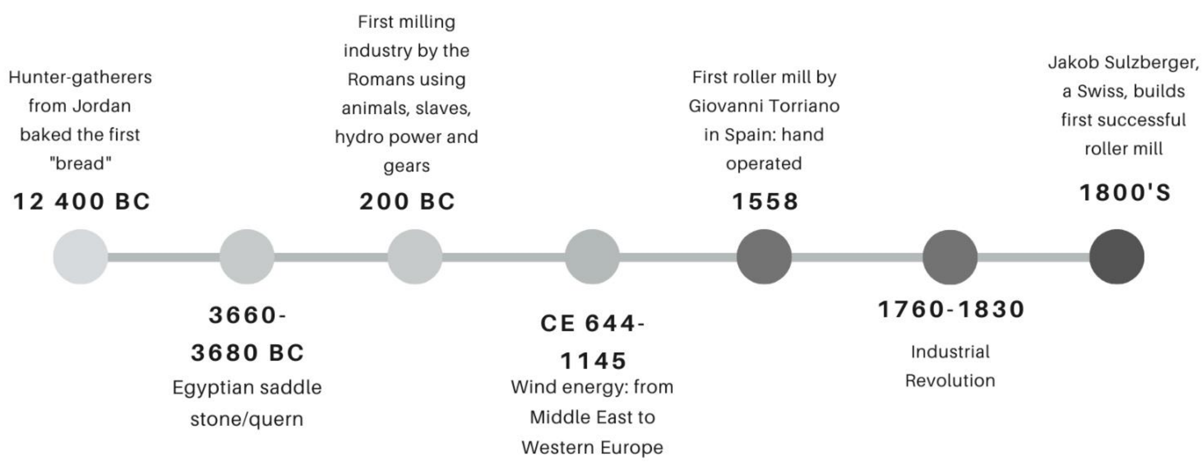

Figure 2.1 Timeline of the development of the wheat flour mill.

An ancient Egyptian tomb dating back to 3660-3680 BC contained a saddle stone and illustrations of flour sieves made from papyrus or horsehair to separate the whole wheat flour meal (Arendt & Zannini, 2013). A saddle stone has a curved stone base whereupon the desired wheat kernels, seeds or nuts are placed. A hand-held rock is then moved forwards and backwards to grind the food until a fine powder is achieved (Walker & Eustace, 2016). A *metate* is the South American version of a saddle stone and was still used until late in the 19th century by Mexicans and Guatemalans (Bauer, 1990). Saddle stones eventually led to the Greek hourglass mill and the quern. Querns are conical milling stones and were used around 800 BC. This is the first time a rotary motion was applied to milling and was often animal-powered (Arendt & Zanninni, 2013; Bauer, 1990; Walker & Eustace, 2016). The upper stone is rotated, thus grinding the grain against the stationary bottom stone.

Since around 200 BC the Romans developed a milling industry by making use of animals, slaves, hydro power and gears to run a mill. By 19 BC, hydro power with gear mechanisms were effectively used by Romans in the form of water mills. Wind energy was being utilized in the Middle East, specifically the Iraqi region, by CE 644 and then eventually moved to Western Europe by CE 1145. The invention of the steam engine by James Watt in 1769 led to the decrease of wind and water mills, especially in areas that did not have access to these natural resources (Walker & Eustace, 2016).

A French miller, Pigeaud, developed the method of gradual reduction of flour in the 16th century (Walker & Eustace, 2016). He did this by milling flour several times with reducing gaps between the stones and then sifting between each step, thus creating a high-quality flour. Austrian and Hungarian mills adopted this approach, leading to about ten products of different qualities being created (Bauer, 1990). From the late 18th to 19th century, several new machine parts and steps were created. This included French and Hungarian purifiers, the patenting of the brush sifter by an Englishman in 1765, plansifters in the 1880s and the first automatic mill (because of the new conveyor belt) in 1785 in the USA (Walker & Eustace, 2016). Automation was driven by the need to supply a single quality product for the entire market (Bauer, 1990).

The first roller mill was designed by Giovanni Torriano in Spain in 1558. The mill was hand operated and comprised of a corrugated cone inside a corrugated shell. With the Industrial Revolution in full swing in Britain (1760-1830), and the need for bread (and thus wheat) rising, global importing escalated. The British climate was suited for soft wheats, however hard wheats (which is normally used for bread) was cultivated in overseas colonies and then shipped to Britain (Bauer, 1990). With the new hard wheats being imported, traditional stone mills were inefficient and produced a much lower flour yield than roller mills. Thus, along with the greater control and efficiency thereof, roller mills gradually replaced stone mills.

A series of less successful attempts to better the design of the roller mill followed until finally, a Swiss engineer called Jakob Sulzberger was able to build a successful roller mill in the 19th century. This mill consisted of rollers to break the wheat kernels and a stone mill to reduce the flour. The rollers were made from steel or porcelain; however, the porcelain broke down much quicker and had to be replaced more often (Walker & Eustace, 2016). The efficiency of roller mills ensured that stone mills were eventually replaced and are still the most common form of mill used today.

2.3 Stone milling

Stone mills are composed of two stones: one fixed and one revolving, with a single grain stream between them (Gélinas *et al*., 2004; Palpacelli *et al*., 2007; Posner & Hibbs, 2011). These stones can be made from granite, emery or flint. Stone mills make use of a combination of shear, compression and abrasion forces to crush the entire wheat kernel and grind it to a smaller particle size, resulting in a theoretical 100% whole wheat flour extraction rate (Kihlberg *et al*., 2004). Modern stone mills can be made from composition stones which are attached to metal plates (Posner & Hibbs, 2011). These mills may also contain sifting cylinders which produce a coarser flour used by smaller bakeries.

Stone milling is mostly done on a small scale, as it is often used to mill specialised wheats such as local, organic or ancient grains, which are produced in much smaller quantities and are popular amongst artisan bakeries (Cappelli *et al*., 2020a; Gélinas *et al*., 2009; Kihlberg *et al*., 2004). Despite the dominance of larger companies in the milling and flour industry, local, regional and home milling practices are undergoing a resurgence of interest due to being economically viable (Ross & Kongraksawech, 2018). The single stream stone mills used in these operations may include small commercial stone mills that do not require the same financial resources to establish as a commercial roller mill. The flour produced from these mills are unsifted whole wheat flour, and the outcome of the quality of the product could be influenced by the miller and the settings of the mill.

Stone mills can be distinguished by movement of the stones, i.e. a fixed top stone (FTS) or a fixed bottom stone (FBS) (Unal & Sacilik, 2011). The FTS mill is also called a horizontal stone mill because of the bottom stone's horizontal movement. The FBS has a top stone that moves horizontally and vertically during milling, therefore it is also known as a vertical stone mill.

2.3.1 White stone milled wheat flour

Previous studies have claimed it is not possible to produce a white stone milled flour because the bran, which has a high ash content, is distributed evenly throughout the flour due to the entire wheat kernel being crushed to a flour (Gèlinas *et al.*, 2004). Despite this, two studies have produced a white stone milled flour in order to analyse the mycotoxin content (Palpacelli *et al*., 2007) and the effect of tempering and stone rotational speed on an ancient wheat flour's rheology, particle size and bread characteristics (Cappelli *et al.*, 2020a). To produce a white stone milled flour, the whole wheat flour was passed through 180 µm sieve in order to separate any bran and germ particles from the endosperm (Cappelli *et al.*, 2020a). Alternatively, the flour can be passed through a separator sieve system (Palpacelli *et al*., 2007). Very little is known about the physicochemical, functional and structural properties of white stone milled flour, as most studies focussed on the traditional whole wheat stone milled flour.

2.4 Stone mill settings

Stone milling is a subjective process (Gèlinas *et al.*, 2004; Ross & Kongraksawech, 2018). A skilled and experienced miller can replicate the flour quality; however no universal standard method or optimal settings standards are available. The miller can adjust the stones and the settings of the mill according to the required particle size distribution and quality, but this is dependent on the type of wheat and the model and make of the mill used (Posner & Hibbs, 2011).

The capacity of a stone mill and the particle size of the flour is dependent on the diameter of the stones, the rotation per minute (rpm) and the power of the motor driving the stones (Posner & Hibbs, 2011). The diameter of modern millstones varies from 13.5 cm to 160 cm, which in turn influences the capacity of the mill from 6.8 kg/h to 600 kg/h. A large-scale stone mill in Egypt, which consists of only stones and sifting cylinders, runs at a capacity of 200 t/24 h or approximately 8.33 t/h (Posner & Hibbs, 2011).

Optimal mill settings (such as milling speed, distance between stones and feed rate) have been addressed in previous studies to a limited extent.

2.4.1 Millstone dressing

The dressing of the mill stones are the cut grooves or furrows that are found on the surface of the stone (Posner & Hibbs, 2011). They play an important role in stone milling as they allow the milled material to move outwards in a radial movement. The furrows need to be regularly sharpened to ensure efficiency (Gèlinas *et al.*, 2004). The stones can be dressed in two ways: the 'quarter' dress and the 'circular'/'sickle' dress (Unal & Sacilik, 2011; Figure 2.2).

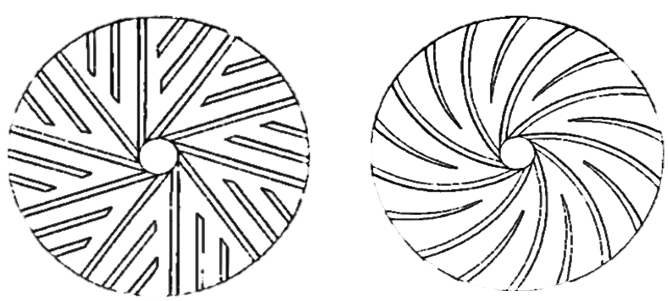

Figure 2.2 Two types of dressing for mill stones: 'quarter' (left) and 'sickle' (right) (Unal & Sacilik, 2011).

Stone abrasiveness significantly decreases after 300 h of milling (Gèlinas *et al.*, 2004). The stones' furrows wear down, resulting in a decreased efficiency in separating the coarse bran from the flour. Therefore, it is advantageous to the miller to service the stone mill regularly to ensure optimal product quality.

2.4.2 Aperture and feed rate

Decreasing the distance between the two stones of a mill, also known as the stone aperture, may affect the quality of a stone milled flour (Gèlinas *et al.,* 2004). The aperture settings of a stone mill can be described as either tight (the maximum stone pressure that allowed movement during milling) or loose (the maximum spacing that allowed the wheat to be milled). The whole wheat flour yield (with a particle size below 250 µm) increased from 67% to 82% when the aperture of the stone mill was reduced to tight, as well as causing the ash content to be higher than when it was at the maximum aperture. The tightening of the stones also resulted in less flour granulation, a lower protein content and more starch damage and higher water absorption. However, simply stating the aperture as 'tight' or 'coarse' is not very precise nor replicable.

The findings of Gèlinas *et al.* (2004) corroborate with those of Ghodke *et al*. (2009). The feed rate (0.21, 0.63, 1.05 min/200 g grain), millstones' aperture (2, 3, 4 mm) and the grain moisture content (8.6, 14.3, 20%) can be adjusted to influence the starch damage content, water absorption, tear force and chapatti texture (chapatti is unleavened flat bread made from whole wheat flour) (Ghodke *et al*., 2009). Aperture had the largest effect on the amount of damaged starch, whereas the chapatti's tear force was mostly influenced by the aperture and moisture content. By tightening the stones and increasing the feed rate, the starch damage of the flour increased, resulting in a higher water absorption of the dough. It was also found that the slowest feed rate resulted in the stickiest dough with the lowest starch damage. By increasing the moisture content of the grain before milling, the tear force decreased, and the chapatti was softer.

2.4.3 Stone rotational speed

The optimal stone rotational speeds (along with the tempering conditions) were determined from 173, 260 or 346 rotations per minute (rpm) for white stone milled flour (Cappelli *et al.,* 2020a). The stone rotational speed affected the mill's productivity, flour yield and specific energy consumption, but not the particle size of the flour. By increasing the rotational speed, the time it took to mill the wheat sample decreased. The optimal mill productivity and lowest specific energy consumption was achieved when the rotational speed was at the highest (346 rpm) and the lowest tempered moisture content (11%).

2.5 Tempering of wheat before milling

Pre-treatment of wheat (tempering or conditioning) before roller milling is essential (Delcour & Hoseney, 2010; Kweon *et al.*, 2009). By adding water to dry wheat before milling, the endosperm becomes softer and the bran tougher and more plastic. This causes the bran to not splinter and distribute throughout the flour and makes the endosperm easier to mill, thus assisting in effective separation. Depending on the hardness of the wheat, the desired moisture level may vary. Hard wheats are usually tempered to 15-16% and soft wheats to 14% moisture before roller milling (AACC International, 1999). Other factors that might also influence the effectiveness of tempering (excluding the tempering temperature and the desired moisture content level) is the duration, wheat cultivar, initial wheat moisture content, kernel size and the kernel temperature before adding water (Posner & Hibbs, 2011).

According to Gèlinas *et al.* (2004), wheat intended for stone milling should not be tempered as the softened grain would stick to the furrows or grooves of the mill, causing blockages and ineffective milling. The crushing of the entire wheat kernel during stone milling causes bran to be found throughout stone milled flour, even once passed through a sieve. In contrast, Cappelli *et al*. (2020a) found that tempering wheat before stone milling could be used as a mechanism to optimise the breadmaking process. High white flour yields were obtained, however the optimal white flour yield (73.3–77.8%) was achieved when wheat was tempered to 13% and 15%, compared to 11% and 17% (71.1–74.8%). This was one of the few studies that investigated white stone milled wheat flour (Type 00 flour with an ash content of 0.48 g/100 g flour). However, it was also found that the higher the moisture content of the tempered wheat kernels, the longer the milling time and the lower the mill's productivity. With an increase in the tempered wheat's moisture content there was also a significant effect on the farinograph water absorption (decreased) and the alveograph P (decreased), L (increased) and P/L (decreased) values. The specific volume, crumb specific volume and crumb moisture was also influenced; however not the crust moisture. Cappelli *et al*. (2020a) thus concluded that tempering to 13% moisture content was the best choice for stone milling and breadmaking performance.

The findings of Cappelli *et al*. (2020a) correlates with a study investigating the optimal tempering of wheat kernels for roller milling. Warechowska *et al.* (2016) studied the effect of various moisture contents on four wheat cultivars by tempering to the following levels: 12%, 14%, 16% and 18%. It was found that by increasing the moisture content before milling, the amount of specific grinding energy needed to mill also increased, which concurs with Cappelli *et al*. (2020a). Furthermore, Warechowska *et al*. (2016) also recorded the influence of these tempered wheat moisture content levels on several other factors such as the flour extraction yield, flour quality, protein content, baking quality, water absorption and particle size distribution. The higher the moisture content of the tempered wheat, the lower the flour extraction yield and ash content, thus producing a white flour with a higher flour quality. Ash content is essential when looking at the flour quality as it represents how well the bran separated from the endosperm, thus a lower ash content represents a more refined white flour. The farinograph water absorption levels increased and the protein content of the flour decreased with increasing tempered moisture content of the wheat. In turn this influenced the gluten content (decreased) and the dough development time (also decreased), which then caused an increase in the sorption capacity.

2.6 Mechanical damage

The amount of mechanical damage to the starch in flour depends on the wheat milling process, which is in turn influenced by the wheat hardness and the technique used (Barrera *et al*., 2013; Yu *et al*., 2015). Softer wheat kernels will undergo less starch damage than hard wheat kernels, regardless the milling method and conditions (Posner & Hibbs, 2011). Hard wheat fractures differently than soft wheat during milling. Intercellular fractures occur when hard wheat is milled, forming larger endosperm particles due to the vitreous structure, whereas soft wheat forms smaller particles when the intracellular fractures occur. The reason for this is that less energy is required to split and extract flour from a soft wheat than a hard wheat, thus there is less damage to the starch molecules (Yu *et al*., 2015). Damaged starch should be similar within the same wheat cultivar, however several factors during milling, such as the milling speed, feed rate and distance between the grinding mechanisms, may influence it (Kihlberg *et al.*, 2004).

Starch damage is an essential quality parameter of wheat flour and occurs when smaller starch particles are fractured from a larger starch granule by breaking the hydrogen and covalent bonds between the molecules (Ma *et al*., 2016; Gibson *et al.*, 1992). Starch damage influences the bread production process as it is necessary for the hydration of the bread dough and the fermentation thereof. High levels of starch damage are directly related to better hydration of the flour when water is added. This leads to a gel-like texture being formed and an increased dough extensibility. Because starch granules are storage mechanisms for energy, smaller starch particles better assist yeasts' bubble formation (Ma *et al*., 2016). The level of starch damage also affects the crumb texture and the dough colour (Gibson *et al.*, 1992). However, too much starch damage can also negatively influence your breadmaking process and final product quality. Excessive starch damage leads to increased enzymatic action of α-amylase during the proofing and baking steps. This may cause the bread's crumb to be sticky and difficult to slice, as well as poor loaf volume and a red crust (Gibson *et al.*, 1992; Ma *et al*., 2016).

2.7 Effect of the milling method and temperature on physicochemical composition of flour

The heat generated during the milling process is caused by the frictional energy between the stones and the grain (Posner & Hibbs, 2011). It is influenced by the material load, stone rotational speed, dwell time and the distance between the stones. Prabhasankar & Rao (2001) indicated that stone mills reached a higher temperatures (90°C) than roller mills (35°C). Plate and hammer mills were respectively 85°C and

55°C. The higher temperatures of stone mills reflected the grinding severity of the mill, resulting in the whole wheat flour having a higher level of starch damage and an altered chemical composition. The stone and plate mills (the two hottest mills) produced flour with the greatest starch damage, protein degradation and amino acid loss, as well as a lower free lipid count and linoleic acid (unsaturated fatty acid) level (Prabhasankar & Rao, 2001). Prabhasankar & Rao's (2001) statistical analysis regarding proteins and fats were not appropriate and were also lacking information on other nutritional constituents such as polyphenols, but despite this, the higher temperature of stone mills cannot be denied.

Ross & Kongraksawech (2018) argued that the levels of starch damage and flour particle size may be a better indication of the grinding severity of a mill than just the temperature. The Osttiroler stone mill produced flour with the coolest temperature (32.1°C) compared to the highest temperatures achieved by the Wonder metal pin mill (51.4°C) and the Perten hammer mill (49°C). The flour temperature of the Brabender Quadramat Senior roller mill (32.3°C) was not significantly different from the Osttiroler stone mill, however the Hawos conical stone mill (40°C), SAMAP conical stone mill (39.5°C) and Meadows stone mill (36.6°C) produced flour that was warmer than the Osttiroler stone mill, but still cooler than the hammer and metal pin mills. These temperatures (up to the maximum of 51.4°C) did not affect the protein and gluten quality. The Osttiroler stone mill had the lowest temperature and longest milling duration, as well as the highest levels of starch damage, a finer particle size and good baking performance. The long dwell time (or milling duration) recorded during milling using the Osttiroler stone resulted in desirable flour properties such as the highest starch damage and high water absorption. The particle size of the flour also influenced other properties such as the Rapid Visco Analyser (RVA) peak time and final viscosity, as well as the loaf volume and farinograph development time and stability. This stone mill produced the finest flour (which was the same particle size as the laboratory scale Brabender roller mill and the hammer mill). The fineness and the high level of starch damage made this stone milled flour the superior flour, according to Ross & Kongraksawech (2018). The findings of Liu *et al.* (2015) corresponded with those of Ross & Kongraksawech (2018) as whole wheat stone milled flour in this study had a significantly higher water absorption than roller and hammer milled flour samples and came only second to the ultra-fine mill. The whole wheat stone milled flour also had significantly lower RVA viscosity parameters than the roller milled flour (Liu *et al.*, 2015).

The rate of increase in flour temperature decreases after 3 000 rpm because the dwell time of the grain on the stone is shorter, thus the flour is only briefly in contact with the high temperatures of the stones (Ross & Kongraksawech, 2018). The lower the stone rotational speed and tangential velocity, the smaller the temperature increase (Cappelli *et al*., 2020a; Ross & Kongraksawech, 2018). The ventilation within the mill, stone diameter and the stone dressing influence the maximum temperature of the mill (Ross & Kongraksawech, 2018).

Furthermore, in a study by Di Silvestro *et al*. (2014), the stone mill had a higher temperature (60°C) than the stone water mill (30°C). This study did not characterise the stone and water mill sufficiently, as the water may not have had an effect on the lower temperature of the stone water mill, nor may it have been the reason for the lower starch damage.

2.7.1 Particle size distribution

The findings of Kihlberg *et al.* (2004) regarding particle size distribution of stone milled flour contrasted with previous studies (Cappelli et al., 2020a; Gélinas *et al*., 2004; Palpacelli *et al*., 2007). Kihlberg *et al*. (2004) indicated that the particle size of stone milled flour was smaller, whereas roller milled flour has a large volume of very small particles and very large particles. In contrast, studies indicated that stone milled flour has either a very coarse or large particle size (Palpacelli *et al*., 2007), or very fine or small particle size if the stone aperture is correctly adjusted (Gélinas *et al*., 2004; Ross & Kongraksawech, 2018). The tighter the stone adjustment, the smaller the flour particle size and the higher the ash content of a whole wheat stone milled flour (Gélinas *et al*., 2004; Islam & Matzen, 1994). The largest particles (>250 µm) in this study had a high ash content (i.e. the most bran), but even the smaller particles (≤212 µm) still had a similar amount of bran, which indicated that sifting was not an effective method to separate the bran from the rest of the stone milled flour (Gélinas *et al*., 2004). By tightening the stones, the flour yield, damaged starch and water absorption increased, but the protein content decreased. The amount of bran particles in the coarsest flour stream (>250 µm) and damaged starch in the finest flour (<105 µm) resulted in the stone milled flours having a higher water absorption.

A study by Cappelli *et al.* (2020) investigated the particle size distribution of a white stone milled flour. The stone milled flour sample had a trimodal curve with peaks at 0.1-1 µm, 20-30 µm and 200-300 µm (Figure 2.3). The two highest peaks indicated that the highest volume particles had a size of 20-30 µm and 200-300 µm.

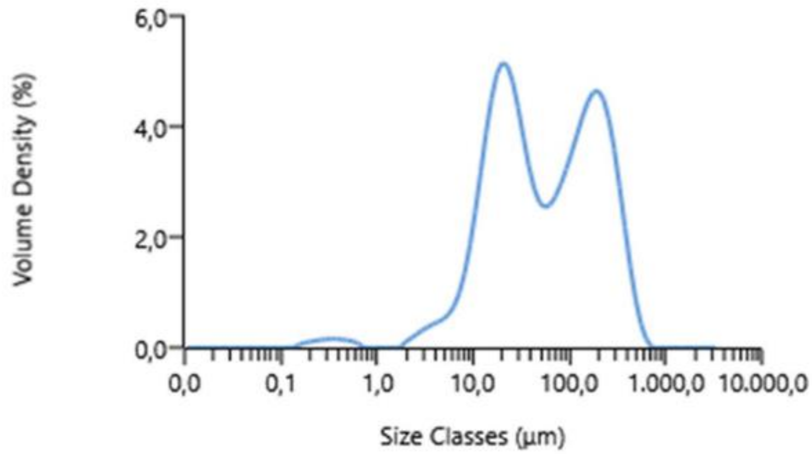

Figure 2.3 Particle size distribution of a white stone milled flour (Cappelli *et al*., 2020).

2.8 Effect of stone and roller milling on sensory characteristics of whole wheat bread

The previously mentioned study by Kihlberg *et al*. (2004) also contrasted with that of Ross & Kongraksawech (2018), Prabhasankar & Rao (2001) and Liu *et al.* (2015), specifically regarding the higher starch damage and water absorption of whole wheat flour produced from stone mills compared to the roller mills. Kihlberg *et al*. (2004) determined that whole wheat roller milled flour had a higher starch damage, SDS (sedimentation volume), farinograph water absorption, dough development and stability than stone milled flour. The flour properties (such as the damaged starch content) and the particle size distribution greatly influenced the sensory characteristics of the whole wheat bread. The high falling number (>300 s) of the stone milled flour was reflected in the lower juiciness attribute. The stone milled flour, which was more homogenous in particle size than the roller milled flour, caused the bread crumb to be more deformed and the bread texture to be of lower quality. Alternatively, the roller milled whole wheat flour had more small and large bran particles, which resulted in the bread loaf being more compact. Breads baked from roller milled flour had a stronger wheat aroma and were sweeter and juicier. This may be due to the larger bran particles in the roller milled flour which caused the bubble structure to be smaller and denser, as it disrupts the cell walls and destabilises the gas bubbles. The other distinguishable sensory characteristics of the stone milled flour was an increase in the saltiness, intensity of the aftertaste, cereal aroma and flavour, as well as an intense roasted cereal taste, crispy crust and crust aftertaste. The reason for the stronger taste has to do with the smaller bran particles found within the stone milled flour, thus promoting the intensity of the flavour. Bran consists of various phenolic compounds which are derived from benzoic acid, such as phytates, folates and sterols. This study did not record any temperatures during the milling process nor information regarding the stone mill (other than 'commercial'), however the breads produced from stone milled flour had a higher crispiness and roasted cereals taste attributed to the high temperature reached by this stone mill. This is a possible reason why the findings might contrast with what was found in other studies regarding the mills and chemical compositions of the flour.

From the varying results of different studies, it can be said that the different stone mills have different effects on the flour that is produced. Not all stone mill flours can be classified as having the same particle size distribution, levels of damaged starch and water absorption levels as the differences in the mill's settings, milling process and temperatures achieved vary greatly depending on the miller and the mill's make and model.

2.9 Combination of stone and roller milling

A combination of stone and roller mills are used to produce a product with acceptable quality and flour yield, whilst maintaining the marketing advantage associated with the terms 'stoneground' or 'stone milled' (Doblado-Maldonado *et al*., 2012; Posner & Hibbs, 2011). The wheat kernel is first broken open by the stone mill (with the plates far apart to ensure minimal heat generation) and then put through the roller mill to be reduced to a flour. Further studies are needed to ascertain more about this milling process, as no study focuses on this nor the effect it may have on the flour compared to roller or stone milling.

2.10 Effect of stone milling on stone ground wheat meal nutrition

2.10.1 Fibre, micronutrients and elements

It is a common belief amongst stone millers that stone milled flour is more nutritional than roller milled flour (Guerrini *et al*., 2019). Whole wheat stone milled flour's major elements and micronutrients content was unaffected by the stone milling process when compared to the grain it was milled from (Albergamo *et al*., 2018). Major elements (such as sodium, magnesium, potassium, calcium and phosphorous) and micronutrients (such as manganese, iron, copper, zinc, selenium, molybdenum and cobalt) remained unaffected when the grains were stone milled to a whole wheat flour, as none of the grain components were removed. Another study by Ficco *et al.* (2016) corroborates this as it was found that total fibre and carotenoids, as well as anthocyanins (found in pigmented grain's pericarp), were better maintained in whole meal stone milled flour than refined roller milled flour. However, non-essential and toxic elements (such as arsenic, nickel, cadmium, lead, chromium and vanadium) in whole wheat flour were also not affected by stone milling (Albergamo *et al*., 2018). On the contrary, roller milling, which removes the contaminated outer layers of the wheat kernel, showed a reduction in the concentration of these elements in refined wheat flour (Cubadda *et al*., 2003; Cubadda *et al*., 2009).

2.10.2 Mycotoxins

Stone milling may lead to a 40-50% reduction of mycotoxins (vomitoxin and zearalenone) in white wheat flour (Palpacelli *et al.*, 2007). The mean vomitoxin (170 ppb) and zearalenone contents (6 ppb) of the stone milled flour were lower than that of roller milled flour (360 ppb and 13 ppb, respectively). This was confirmed by the analysis of randomised commercial stone and roller milled flour, which indicated that the mean vomitoxin of the stone and roller milled flour were respectively 245 ppb and 945 ppb and the zearalenone 1.7 ppb and 6.0 ppb. The stone milled white flour in this study was achieved by putting the whole wheat flour through a separator sieve set. The larger reduction of mycotoxins in stone milled flour was attributed to the stone milling system used in this study as it had a trimming machine with an aspirator. This resulted in the external layers of the wheat kernels, which contain the highest concentration of mycotoxins, being partially removed. The roller milling process, specifically the reduction steps, resulted in mycotoxins being distributed throughout the flour.

2.11 Conclusions

Stone milled flour is associated with being more nutritional than roller milled flour, and there is often a marketing advantage associated with it. Results regarding the heat generated by stone mills and mill settings (such as aperture, feed rate, rotational speed) vary vastly, leading to discrepancies in properties such as particle size distribution and starch damage. This may be due to the stone mill process, settings and designs varying vastly between different models, as well as millers following their own methods for their respective mills.

Despite the recent resurgence in the popularity of stone mills, research falls short on several aspects regarding stone milling process. Very little is known about combination milling, and only a few studies focus on important aspects such as nutrition, sensory and physicochemical properties of whole wheat stone milled flour. Further studies on white stone milled flour is needed, as modern commercial stone mills are producing this product. Prior research has not thoroughly investigated the physicochemical, structural and functional properties of white stone milled flour.

2.12 References

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CHAPTER 3

Effect of stone and roller milling on the physicochemical, functional and structural properties of white wheat flour

Abstract

The effect of stone and roller milling on the physicochemical, functional and structural properties of wheat flour was analysed. As very little prior research is available on the properties of refined white stone milled flour, the aim of this study was to provide a baseline on which future studies could build on. Stone and roller milled flour samples were produced from two wheat samples, namely a hard commercial cultivar wheat and a hard commercial wheat blend. The results indicated that the milling method often had a larger effect than the wheat type or the combination of wheat type x milling method, and this may be due to the similarity in the hardness of the two wheat samples. The flour yield of the sifted stone milled flour was too low to be economically viable. Sieving whole wheat flour through a 212 µm sieve proved ineffective in removing the bran from the rest of the stone milled flour. The ash content significantly differed ($P \leq 0.05$) from the roller milled samples and was too high to classify the stone milled flour as a 'white bread wheat flour' according to South African wheat flour regulations. The water absorption capacity was significantly higher ($P \leq 0.05$) for stone milled flour than roller milled flour due to the high ash content, smaller particle size ($P \leq 0.05$) and high starch damage levels ($P \leq 0.05$). The higher levels of starch damage were associated with a higher falling number ($P \leq 0.05$) for stone milled flour. The smaller flour particle size and high levels of starch damage affected the alveograph, mixograph and Rapid Visco Analyser (RVA) pasting properties. Scanning electron micrographs (SEM) qualitatively illustrated the starch damage and non-uniformity of the stone milled flour. In conclusion, stone milling proved to have an effect on the physicochemical, functional and structural properties of flour. It was not possible to produce a white stone milled flour in adherence to South African wheat flour regulations using the stone milling method in this study.

3.1 Introduction

Wheat milling is an essential process in the production of wheat products, specifically wheat flour (Delcour & Hoseney, 2010). The two predominant milling methods used to produce a wheat flour are roller milling and stone milling (Doblado-Maldonado *et al*., 2012). Roller mills consist of sets of corrugated and smooth metal rollers that entail various systems (namely the break, sizing, reduction and tailings systems) and reciprocating sieves that separate the bran and germ from the endosperm to produce a white wheat flour (Delcour & Hoseney, 2010). It makes use of shear, scrape and crush actions to remove the endosperm from the germ and the bran. The endosperm is then reduced in size and selected flour streams are combined to produce a white wheat flour (Posner & Hibbs, 2011). Wheat tempering takes place prior to roller milling to ensure that the endosperm is softened, and the bran does not fragment and disperse into the flour (Delcour & Hoseney, 2010). Contrasting studies indicate that tempering before stone milling can be used as a mechanism to increase the white flour yield and produce a finer flour with more starch damage (Cappelli *et al*., 2020), or it may result in blockages of the stone mill's furrows due to the softened grain (Gèlinas *et al*., 2004).

Stone milling produces a stone milled (also referred to as 'stone ground' or 'stoneground') wheat flour by grinding a single wheat grain stream between one fixed and one revolving stone (Doblado-Maldonado *et al.*, 2012; Gèlinas *et al*., 2004). It is a subjective process that is reliant on the experience of the miller (Ross & Kongraksawech, 2018). Stone milled flour is often perceived as a niche artisanal food product and has a distinct marketing advantage associated with the term 'stone ground' on account of consumers seeing it as nutritionally superior to roller milled flour (Di Silvestro *et al*., 2014; Guerrini *et al*., 2019). In recent years, stone milled flour has been garnering attention as a healthier and more flavoursome flour than other commercially available roller milled flour. On the other hand, previous studies indicated that the grinding severity and high temperatures generated during stone milling results in higher levels of protein degradation and damaged starch, as well as a lower free lipid content compared to other milling methods (Prabhasankar & Rao, 2001). The flour particle size, damaged starch and heat generated during stone milling are indicators of the grinding severity of the process (Prabhasankar & Rao, 2001; Ross & Kongraksawech, 2018). Stone milling typically produces a whole wheat flour containing all the parts of the wheat kernel and with an extraction rate of 100% (Kihlberg *et al*., 2004). Whole wheat stone milled flour often has a higher water absorption due to increased levels of starch damage and bran content (Gèlinas *et al*., 2004; Prabhasankar & Rao, 2001; Ross & Kongraksawech, 2018). The high starch damage of whole wheat stone milled flour influences the pasting properties by having a higher peak and final viscosity than roller milled flour (Ross & Kongraksawech, 2018). A distinctive study by Kihlberg *et al*. (2004) found that whole wheat stone milled flour had a lower starch damage content, which is in contrast with the abovementioned studies. The lower levels of starch damage of stone milled flour resulted in a lower water absorption and higher falling number than roller milled flour, leading to a deformed crumb and inferior bread texture.

Very little is known about the properties of refined white stone milled flour as previous studies focussed on the mycotoxin content (Palpacelli *et al*., 2007), or the effect of tempering and stone rotational speed on predominantly the flour yield, particle size and farinograph and alveograph properties (Cappelli *et al*., 2020). These studies indicated that white stone milled flour is produced by passing the whole wheat stone milled flour through a sieve. Another study contrasted with this and indicated that it is not possible to separate all the bran from the endosperm, as the stone milling process does not contain a separation step of the bran and endosperm as with roller milling, resulting in the entire wheat kernel being ground to a similar flour particle size and the bran being distributed throughout the flour (Gèlinas *et al*., 2004). Stone millers often address this challenge by making use of a process called 'combination milling', or a combination of stone and roller milling (Doblado-Maldonado *et al.,* 2012; Posner & Hibbs, 2011). The millstone aperture is large enough to minimise heat generation and to crack the wheat kernel open before being reduced to a flour using a roller mill.

The aim of this study was to determine the physicochemical, functional and structural characteristics of white roller and stone milled wheat flour produced from two wheat samples, namely a hard commercial cultivar and a hard commercial blend.

3.2 Material and methods

3.2.1 Whole wheat grain sampling and analyses

Wheat samples

Two wheat samples were provided by Sasko Research and Development (Essential Foods, Division of Pioneer Foods (Pty) Ltd., Paarl, South Africa) and Sensako (Pty) Ltd. (Bethlehem, South Africa). Upon receival, samples were stored at Sasko in sanitised, sealed containers to ensure sample integrity until analyses could be conducted. The wheat samples consisted of an imported hard commercial blend from Canada and a South African hard commercial wheat cultivar (SST 8154). These samples shall henceforth be referred to as the Blend and Cultivar wheat samples, respectively.

The samples were thoroughly cleaned by removing any foreign objects, broken kernels and other impurities using a Carter Day Dockage Tester (Carter Day International, Minneapolis, MN, USA). Each wheat sample was subsequently mixed by pouring it through the Boerner Divider (Seedburo Equipment Co., Chicago, IL, USA) three times and dividing it into six batches (three for stone milling and three for roller milling). The batches intended for roller and stone milling were each 3.5 kg and 8.5 kg, respectively.

Wheat kernel characterisation: moisture content, protein content, hardness index, kernel weight and kernel diameter

The moisture and protein contents of the wheat kernels were determined using the Perten Instruments Inframatic 9500 NIR Grain Analyser (Hägersten, Sweden). Wheat kernels were poured into the instrument's funnel and measured in duplicate.

The Single Kernel Characterisation System (SKCS) was used to determine the wheat kernel texture according to AACC method 55-31.01 (AACC International, 1999h) with the SKCS model 4100 (Perten Instruments, Hägersten, Sweden). This provided the following information regarding the wheat samples: hardness index (HI), kernel weight (mg), moisture content (%) and kernel diameter (mm). The wheat can be classified into the different hardness categories according to the hardness index (Table 3.1).

Table 3.1 Guidelines for hardness index (AACC method 55-31.01)

Category	Hardness Index (HI)
Extra hard	90 +
Very hard	81-90
Hard	65-80
Medium hard	45-64
Medium soft	35-44
Soft	25-34
Very soft	10-24
Extra soft	Up to 10

3.2.2 Wheat tempering

Tempering of all the wheat samples to a desired moisture content of *ca*. 15.5% took place prior to both stone and roller milling. The wheat samples were tempered according to the AACC method 26-95.01 by adding the calculated (Eq. 1) amount of distilled water (dH_2O) to the wheat kernels (AACC International, 1999b). The tempering drums with the wheat and water were rotated for 1 h to ensure a uniform distribution of the water. After 18 h the moisture content of the wheat was measured using the Perten Instruments Inframatic 9500 NIR Grain Analyser (Hägersten, Sweden) to determine if the tempering process was successful and the desired moisture content was reached.

$$Weight\ of\ water\ to\ add\ (g) = \left(\frac{100 - \text{original moisture (\%)}}{100 - \text{desired moisture (\%)}}\right) \times sample\ weight(g) \qquad \text{(Eq. 1)}$$

3.2.3 Wheat milling

Roller milling

Roller milling took place at Sasko Research and Development and made use of an experimental Bühler MLU-202 six stream mill (Bühler Co., Uzwill, Switzerland). Before milling took place, the mill was run empty for 30 min and then checked if it was clean. A 100 g warmup sample was run through the mill to ensure optimal milling conditions. The temperatures of the front and back rollers of the mill, as well as the temperature of the flour that exited the mill, were measured using a Xuilee GM 320 Infrared Thermometer. The bran and pollard and the six white flour streams were collected and weighed. The white wheat flour streams were then mixed with a roll container to ensure homogeneity. The samples were stored in plastic bags and placed in sealed containers at ambient temperature until further analysis could take place.

Stone milling

A EuropeMill 600W Industrial Horizontal stone mill (ENGSKO United Milling Systems, Randers, Denmark) from Gideon Milling (Cape Town, South Africa) was used. The motor power was 7.5 kW, stone diameter 1.2 m and the grinding mill rotations per minute (rpm) set at 480 rpm. The mill was first run on empty for 10 min to ensure any remaining product moved through the mill and could be discarded, as well as obtaining optimal milling conditions. A sample of 5 kg of the wheat sample that was to be milled was then run through the mill and discarded to decrease the risk of contamination from previous flour that had been in contact with the mill (Kihlberg *et al.*, 2004). The external temperature of the mill (it was not possible to directly measure the temperature of the stones due to the metal casings) and the temperature of the flour as it exited the mill was measured using a Xuilee GM 320 Infrared Thermometer. In order to obtain a refined sifted flour, the flour was then sifted in smaller batches using a 200 mm diameter horizontal, circular laboratory Sieve Shaker (Scientific Manufacturing cc., Cape Town) with a 212 µm LABOTEC Test Sieve (Clear Edge Filtration SA Pty (Ltd), South Africa). The flour was weighed and stored in plastic bags in airtight containers at ambient temperature until analyses could take place.

3.2.4 Flour yield

Flour yield was calculated as the refined flour's total weight as a percentage of the total weight of the tempered wheat grain before it was milled.

3.2.5 Physicochemical, functional and structural analyses of flour

Moisture content of flour

The moisture content of the wheat flour was determined in duplicate immediately after milling according to the AACC 44-15.02 Air-Oven method (AACC International, 1999c). The flour samples were dried in the CHOPIN EM 10 air oven (CHOPIN Technologies, Villeneuve la Garenne, France) at 130°C for 60 min. The lids were then placed on the samples and they were allowed to cool in the desiccator for 45 min before weighing and moisture content calculation.

Ash content

The ash content of the flour samples was determined in duplicate by employing the ash-rapid (magnesium acetate) method as in AACC method 08-02.01 (AACC International, 1999a). Wheat flour samples (3 ± 0.01 g) and a prepared magnesium acetate solution (5 mL) were added to the pre-ignited ashing dishes. The samples were then placed in the muffle furnace at 700°C and allowed to flame until carbonised, whereupon the oven door was closed, and the samples were incinerated for 45 min. The samples were then removed from the oven and allowed to cool in a desiccator before weighing and ash content calculation (Eq. 2).

$$\%\,Ash = \frac{weight\ of\ ash - weight\ blank}{sample\ weight} \times 100 \qquad \text{(Eq. 2)}$$

Crude protein content of flour

The Dumas combustion method was conducted in accordance with AACC method 46-30.01 to determine the crude protein content (on a 12% moisture basis). This was done in duplicate using the LECO TruMac N Nitrogen analyser (Saint Joseph, MI, USA). The total nitrogen (N) was multiplied by a conversion factor of 5.7 to determine the protein content for the wheat flour (AACC International, 1999d).

Colour

CIELab Colour analysis was performed on the wheat flour samples using the Konica Minolta Spectrophotometer CM-5 (Chiyoda City, Tokyo, Japan). CIELab values were obtained in duplicate to determine the L* (lightness), a* (redness or red-green value) and b* (yellowness or blue-yellow value) with the Observer set at 10 Degrees and the Illuminant at D65.

Particle size distribution

The particle size distribution of the flour samples was determined in triplicate using a PSA 1190L/D laser diffraction particle size analyser (Anton Paar GmbH, Graz, Austria) on the dry mode with a measuring range of 0.1 µm to 2500 µm. The testing parameters were 500 mbar air pressure and the testing duration was 10 seconds per sample repetition. The median particle size (D50) was established, as well as expressing the distributions as volume percentage fraction of the particle size classes.

Gluten analyses

AACC method 38-12.02 was employed to complete the gluten analysis of the wheat flour in duplicate. Flour (10 ± 0.01 g) was mixed and washed using the Perten GM 2200 Glutomatic (Perten Instruments, Hägersten, Sweden) (AACC International, 2000). The duplicates were run at the same time. The samples were allowed to mix for 20 s to produce a dough and then started a 5 min wash cycle. The chambers in which the samples were washed consisted of an 88 µm polyester and 840 µm polyamide screen and screen holder. The doughs were then placed in a Gluten Index Centrifuge 2015 (Perten Instruments, Hägersten, Sweden) and centrifuged at 6 000 ± 5 rpm. The material that had passed through the sieve and the material that had remained on the sieve were separately weighed and recorded as the wet gluten. The wet gluten was then transferred to a Glutork 2020 gluten dryer apparatus (Perten Instruments, Hägersten, Sweden) operated at 150°C and was allowed to dry using a heating cycle of 4 min. Wet gluten content (%), gluten index, dry gluten content (%) and water binding capacity (%) were determined from measurements recorded in this analysis, using Eq. 3-6.

$$Wet\ gluten\ content\ (\%, 14\%\ moisture\ basis) = \frac{total\ wet\ gluten\ (g) \times 860}{100 - \%\ sample\ moisture} \qquad \text{(Eq. 3)}$$

$$Gluten\ index = \frac{wet\ gluten\ remaining\ on\ sieve\ (g) \times 100}{total\ wet\ gluten\ (g)} \qquad \text{(Eq. 4)}$$

$$Dry\ gluten\ content\ (\%, 14\%\ moisture\ basis) = \frac{total\ dry\ gluten\ (g) \times 860}{100 - \%\ sample\ moisture} \qquad \text{(Eq. 5)}$$

$$Water\ binding\ capacity\ (\%) = wet\ gluten\ content\ (\%) - dry\ gluten\ content\ (\%) \qquad \text{(Eq.6)}$$

Starch damage

The damaged starch content of the flour samples was determined according to AACC Amperometric method 76-33.01 using the SDmatic (CHOPIN Technologies, Villeneuve la Garenne, France) (AACC International, 2007).

Falling number

The falling number (FN) was determined in duplicate according to AACC method 56-81.04 (AACC International, 2019) by adjusting the sample weight on a 14% moisture basis using a Perten FN 1500 (Perten Instruments, Hägersten, Sweden). Flour (7 ± 0.05 g flour, 14% mb) was added to the viscotube, after which 25 mL distilled water (at 22-25°C) was added with a pipet. The viscotube was then closed with a rubber stopper and placed in the mixer and mixed 20-30 times. The sides of the tube were scraped down with the viscometer-stirrer before being placed in the boiling water bath for 30-60 s. The instrument would then record the falling number value in seconds. The laboratory's altitude was 120 m, which is less than the stipulated 760 m, therefore no altitude corrections were made.

Consistograph

The AACC method 54-50.01 was followed to determine the water absorbtion capacity (WAC, %) with the Alveolab consistograph (CHOPIN Technologies, Villeneuve la Garenne, France) for each flour sample (AACC International, 1999g). This analysis consists of two parts. During the first part (the constant hydration test), the flour sample (250 g) and prepared sodium chloride (NaCl) solution (calculated according to the flour sample's moisture content) were added to the consistograph's mixing bowl. The dough was mixed for 30 s and then stopped to scrape down the sides of the bowl (within 1 min) to ensure all the flour was incorporated in the dough. The mixing then proceeded until a total of 240 s since the commencement of the test. The hydration level of the sample was produced. The second part of the analysis (the adapted hydration test) uses the hydration level to determine the calculated amount of flour to add to the cleaned mixing bowl. The flour and prepared NaCl solution were mixed, stopped and scraped down according to the same time schedule as the first test. The mixing then commenced until a total of 480 s of mixing time; and the WAC was procured.

Mixograph

Following AACC method 54-40.02, a mixograph (National Mfg Co., Lincoln, Nebraska, USA) was used to determine the dough's resistance to mixing (peak height, mm) and the optimum mixing time (min) (AACC International, 1999f). Duplicate flour samples (35 ± 0.01 g, 14% mb) of each batch was weighed out and placed in the mixograph bowl. Water (determined by the mixograph's software using Eq. 7) was dispensed from a burette into the mixing bowl.

$Y = 1.5X + 43.6$ (Eq. 7)

Where X is the flour protein content (%, 14% mb) and Y is the percentage absorption water. The dough was mixed for 7 min by three rotating and four stationary pins to measure the torque resistance presented by the dough's development.

Alveograph

Alveograph analysis using the AlveoLab (CHOPIN Technologies, Villeneuve la Garenne, France) was performed in duplicate according to AACC method 54-30.02 (AACC International, 1999e). Wheat flour (250 ± 0.5 g) was placed in the alveograph's mixing bowl and the calculated amount of prepared NaCl solution was added to each sample. This was determined by the alveograph's software according to the moisture content of the sample. The alveograph mixed the dough for 1 min and would then stop for 1 min to allow dough to be scraped down from the sides of the mixing bowl. The dough would mix for a further 6 min. Extrusion of the test pieces on the greased receiving plate would then commence. Test pieces were rolled to a uniform height and then cut into a circular shape with the alveograph's specialised cutter. The test pieces were placed on greased resting plates and transferred to the resting chamber (at 25 ± 0.2°C). When the timer reached 28 min since the mixing commenced, the first test piece was placed between two metal plates. Air pressure was used to expand the dough into a bubble and the internal pressure was moderated. The following information regarding the dough's resistance to extension was measured by obtaining five curves per sample: the resistance of the dough deformation indicated by the height of the curve (P, mm); the dough extensibility indicated by the length of the curve (L, mm); curve configuration ratio (P/L, mm) and the deformation energy (W, 10^{-4} J).

Pasting properties

By making use of the Rapid Visco Analyser (RVA) model 4500 (Perten Instruments, Hägersten, Sweden) pasting curves of the flour samples were produced in duplicate according to AACC method 76-21.02 (AACC International, 2017). Flour (3.5 g ± 0.01 g) was weighed out on 14% moisture basis (mb) and then added to the calculated amount of distilled water in the aluminium test canister, which was determined according to the moisture content of the samples. The plastic paddle was moved by hand to ensure that the flour-water suspension had no lumps before it was placed into the RVA. The stirrer was activated, and measurement commenced when the motor tower was pressed downwards, thus allowing the apparatus to run on the pre-programmed Standard Profile 1 (Table 3.2) for 13 min. The following data was gathered: peak viscosity (Vp, cP), breakdown viscosity (Vb, cP), trough viscosity or the minimum viscosity after the peak (Vt, cP), final viscosity (Vf, cP), setback viscosity (Vs, cP), pasting temperature (°C) and the time to the peak viscosity (min). The software that was used to conduct this test was *Thermocline for Windows*™ (version 3).

Table 3.2 Details of the RVA Standard Profile 1

Stage	Standard Profile 1
Initial temperature (°C)	50
Initial holding time (min)	1:00
Heating time (min)	3:42
Maximum temperature (°C)	95
Hold at max temperature (min)	2:30
Cooling time (min)	3:48
Final temperature (°C)	50
Final holding time (min)	2:00
Total test time (min)	13:00

Scanning electron microscopy

Double-sided carbon tape was placed on aluminium stubs. The wheat flour samples evenly coated the carbon tape. The sample was placed in a Leica EM ACE200 Gold Sputter Coater (Leica Microsystems, Germany) to be sputter coated with a *ca.* 10 nm layer of gold. Thereafter the sample was placed in a 50°C incubator for a minimum of 12 h to ensure excess moisture was removed and the sample integrity maintained. The scanning electron micrographs (SEM) were captured using a Zeiss MERLIN Field Emission Scanning Electron Microscope (FE-SEM/FESEM) (ZEISS, Germany) at the Electron Microbeam Unit of Stellenbosch University’s Central Analytical Facility. A Zeiss 5-diode Back Scattered Electron (BSE) Detector (Zeiss NTS BSD) and Zeiss Smart SEM software were used to generate BSE images. The accelerating voltage of the beam during the analysis was 20 kV, the probe current 16nA, the working distance 9.5 mm and the beam current 11nA. Micrographs were obtained with variable magnifications to observe the flour morphology.

3.2.6 Statistical analysis

Statistical analysis was conducted using STATISTICA version 13.6 (StatSoft Inc., Tulsa, Ok, USA). A mixed-model analysis of variance (ANOVA) lme4 package in R was used to analyse the mean differences between the wheat samples, the milling methods and the wheat sample x milling method at a 95 % confidence interval to indicate significant differences, namely a 5% significance level (P≤0.0.5). The data were presented as mean ± standard deviation. The Fisher least significant difference (LSD) test was done to perform the different post hoc analyses.

A principal component analysis (PCA) biplot was created to indicate the correlation between different components that were analysed. This was done using XLSTAT version 2019.1 (Addinsoft, United States of America).

3.3 Results and discussion

3.3.1 Whole wheat grain descriptive results

Hardness index, protein and moisture contents are shown in Table 3.3 and Table 3.4. Grain hardness plays a vital role in milling as it affects the milling yield, flour particle size, tempering requirements and starch damage (Bettge & Morris, 2000; Pasha *et al*., 2010; Posner & Hibbs, 2011). Both wheat samples (Cultivar and Blend) were classified as hard based on SKCS hardness index (HI) (Table 3.3). Milling hard wheat consumes more power and produces a coarser flour with a higher level of starch damage compared to soft wheat (Bettge & Morris, 2000; Pasha *et al*., 2010). The damaged starch absorbs more water than native starch, along with a higher protein content, makes hard wheat more suitable for yeast-leavened bread production than soft wheat. Soft wheat contains more intact starch molecules, less damaged starch and is more suitable for biscuits and pastries. The Cultivar sample had a higher protein content (13.37 ± 0.06%) than that of the Blend wheat (12.6 ± 0%) (Table 3.4). The moisture content results confirmed that the wheat samples were tempered to 15.2-15.7% (Table 3.4).

Table 3.3 Single Kernel Characterisation System (SKCS) results of Cultivar and the Blend wheat samples

Wheat sample	Hardness Index	Kernel weight (mg)	Moisture content (%)	Kernel diameter (mm)
Cultivar	78 ± 12	44.8 ± 8.8	10.7 ± 0.3	2.93 ± 0.33
Blend	76 ± 15	43.1 ± 11.5	12.8 ± 0.4	2.97 ± 0.36

Values are mean ± standard deviation for the 3 batches, measured in duplicate

Table 3.4 Protein content and moisture content results before and after tempering of the Cultivar and Blend wheat samples

Wheat sample	Protein (12% mb*)	Moisture content before tempering (%)	Moisture content after tempering (%)	
Cultivar	13.37 ± 0.06	10.2 ± 0	Roller	15.57 ± 0.06
			Stone	15.15 ± 0.03
Blend	12.6 ± 0	12.0 ± 0	Roller	15.7 ± 0
			Stone	15.42 ± 0.02

Values are mean ± standard deviation for the 3 batches, measured in duplicate
*mb = moisture basis

3.3.2 Flour yield

The flour yield (or extraction rate) is an essential evaluation of the efficiency of the milling process (Delcour & Hoseney, 2010). The flour yields for the roller milled flour for both wheat samples (Cultivar and Blend) were more than double (64.56-65.03%) than that of the stone milled flour (26.29-28-39%) (Table 3.5). The low yield of the stone milled flour indicates that this would not be a commercially viable milling process. The flour loss was too high to ensure efficiency and economical gain. A typical yield for roller milled white wheat flour is *ca*. 72% (Delcour & Hoseney, 2010).

It is advised that wheat intended for stone milling should not be tempered, as the wheat will become too soft and cause blockages of the stones' furrows (Gélinas *et al*., 2004). As all wheat samples in this study were tempered to 15.2-15.7% (Table 3.4), blockages of the stones could have contributed to the low yield. A recent study showed that tempering a weak, ancient wheat to 13% and 15% for stone milling resulted in a higher white flour yield (73.3–77.8%) than when tempered to 11 and 17% moisture content (71.1–74.8%) (Cappelli *et al.*, 2020). This high yield is unexpected taking in consideration that Cappelli *et al*. (2020) used a 180 µm sieve size and the current study used a 212 µm sieve. Several factors could have contributed to the difference in flour yields between the two studies, such as the difference between the stone mill models (e.g. laboratory vs. commercial mill) and efficiency of the sieving process. The stone mill flour yield could potentially be increased by tightening the stones during milling as well as ensuring the stones are abrasive enough (Gélinas *et al*., 2004).

Table 3.5 The flour yield (%) for stone and roller milled Cultivar and Blend flour samples

Milling method	Wheat sample	Flour yield (%)
Stone mill	Cultivar	26.19 ± 0.28
	Blend	28.39 ± 3.90
Roller mill	Cultivar	64.56 ± 0.90
	Blend	65.03 ± 0.25

Values are mean ± standard deviation for the 3 batches, measured in duplicate

3.3.3 Flour temperature and grinding severity

Roller milling relies on the break and sizing systems to separate the bran and germ from the endosperm, whereas during stone milling the entire wheat kernel is reduced in size to produce a flour (Posner & Hibbs, 2011). Stone milling results in significant friction between the wheat and the stones, thus generating a high temperature (Posner & Hibbs, 2011; Prabhasankar & Rao, 2001). Previous studies have indicated that the higher damaged starch levels and finer particle size of stone milled flour has been attributed to the increased grinding severity, which is influenced by the higher milling and flour temperatures and longer dwell times (Ross & Kongraksawech, 2018; Prabhasankar & Rao, 2001). The temperature of the mill is not the sole indicator of grinding severity as this can be influenced by the mill's ventilation systems and the geometry, dressing (furrows) and diameter of the millstones (Ross & Kongraksawech, 2018). The stone milled flour samples in this study had a finer particle size and higher starch damage than the roller miller milled flour, which could indicate an increased grinding severity.

The only comparable temperature between the roller and stone mills was the flour temperature as it exited the mills. The roller milled flour temperature was *ca*. 21-23°C and the stone milled flour was slightly higher at *ca*. 25-27°C. Measuring the temperature of the stone and roller mills posed several challenges, mainly due to the physical and processing differences of the two mills. The stone mill was encased in a metal covering, making it impossible to directly measure the temperature of the stones during production. However, the Bühler roller mill's rollers were possible to measure directly. The stone mill's (metal casing) temperatures were *ca*. 29-32°C. The temperatures of the front and back rollers of the roller mill were *ca*. 26-35°C. These temperatures were much lower than Prabhasankar & Rao (2001), who reported stone and roller mills at respectively 90°C and 35°C, but closer to the temperature of stone mills (32.1-40.0°C) and a roller mill (32.3°C) by Ross & Kongraksawech (2018).

3.3.4 Physicochemical properties

The physicochemical properties of the stone and roller milled flour samples include the moisture content, ash content, protein content, colour analysis and median particle size (Table 3.6). The particle size distribution for the four flour samples is illustrated in Figure 3.1. The gluten analysis, falling number and starch damage is presented in Table 3.7.

Table 3.6 The ash, moisture and protein content (%) as well as the colour analyses and median particle size (µm) of the flour samples

Mixed model ANOVA	Moisture content (%)	Ash content (%)	Protein content (%)	Colour			Median particle size (µm)
				L*	a*	b*	
F values (p values)							
Wheat sample	37.98 (≤0.05)	1.14 (0.30)	282.13 (≤0.05)	85.31 (≤0.05)	4.42 (0.07)	128.93(≤0.05)	15.74 (≤0.05)
Milling method	128.94 (≤0.05)	703.64 (≤0.05)	1208.64 (≤0.05)	3894.93 (≤0.05)	2858.17 (≤0.05)	926.24 (≤0.05)	128.91 (≤0.05)
Wheat sample x milling method	0.28 (0.61)	1.51 (0.23)	38.22 (≤0.05)	21.87 (≤0.05)	4.70 (0.06)	0.22 (0.65)	5.94 (≤0.05)
Mean: Wheat sample							
Cultivar	15.15 ± 0.27^{b}	0.91 ± 0.42^{a}	14.25 ± 1.53^{a}	90.79 ± 2.78^{a}	1.34 ± 0.69^{b}	12.43 ± 0.88^{a}	77.90 ± 12.48^{b}
Blend	15.41 ± 0.26^{a}	0.88 ± 0.47^{a}	13.05 ± 1.09^{b}	89.94 ± 3.23^{b}	1.40 ± 0.75^{a}	11.82 ± 0.84^{b}	84.72 ± 8.93^{a}
Mean: Milling method							
Roller mill	15.04 ± 0.16^{b}	0.47 ± 0.1^{b}	12.41 ± 0.4^{b}	93.24 ± 0.23^{a}	0.68 ± 0.01^{b}	11.31 ± 0.31^{b}	91.07 ± 1.71^{a}
Stone mill	15.52 ± 0.17^{a}	1.31 ± 0.04^{a}	14.89 ± 0.89^{a}	87.49 ± 0.70^{b}	2.06 ± 0.08^{a}	12.95 ± 0.35^{a}	71.56 ± 7.09^{b}
Mean: Wheat sample x milling method							
Cultivar x roller mill	14.9 ± 0.11^{d}	0.51 ± 0.09^{b}	12.79 ± 0.04^{c}	93.45 ± 0.07^{a}	0.68 ± 0.01^{c}	11.60 ± 0.08^{c}	89.75 ± 1.35^{a}
Cultivar x stone mill	15.4 ± 0.05^{b}	1.31 ± 0.03^{a}	15.72 ± 0.04^{a}	88.13 ± 0.12^{c}	2.01 ± 0.04^{b}	13.26 ± 0.16^{a}	66.05 ± 2.02^{c}
Blend x roller mill	15.18 ± 0.06^{c}	0.44 ± 0.11^{b}	12.03 ± 0.03^{d}	93.03 ± 0.06^{b}	0.68 ± 0.01^{c}	11.02 ± 0.02^{d}	92.38 ± 0.68^{a}
Blend x stone mill	15.64 ± 0.16^{a}	1.31 ± 0.05^{a}	14.07 ± 0.34^{b}	86.85 ± 0.26^{d}	2.12 ± 0.08^{a}	12.63 ± 0.05^{b}	77.06 ± 5.83^{b}

Values are mean ± standard deviation of 3 batches in duplicate (wheat sample: n=12; milling method: n=12; wheat sample x milling method: n=6)
Mean values with different superscripts in a column differ significantly (P≤0.05)
Protein expressed as N x 5.7 on a 12% moisture basis

Moisture content

Moisture content (%) plays a vital role in the shelf life and storage of flour. Both the mean wheat sample (Cultivar and Blend wheat) and the mean milling method (stone and roller milling) indicated a significant difference ($P \leq 0.05$) between the moisture contents. The milling method had the largest effect on the moisture content of the flour, as indicated by the largest F-value (128.94) (Table 3.6). The mean moisture content of roller milled flour (15.04 ± 0.16%) was significantly lower ($P \leq 0.05$) than the stone milled flour (15.52 ± 0.17%).

As the moisture content of the flour samples are higher than 14%, the kernel's metabolism may be increased and the growth of microorganisms, fungi and mycotoxins may be encouraged (Cardoso *et al*., 2019). According to South African wheat product standards (Department of Agriculture, Forestry and Fisheries, 2017), the maximum moisture content for flour is 14%. All the flour samples produced in this study exceed this limit, however this is something that can be corrected in an industrial storage and blending operation using pneumatic flash dryers (Posner & Hibbs, 2011).

Ash content

The ash content reflects the bran contamination in a flour, as it is usually higher in the outer layers of the wheat kernel than the inner layers (Delcour & Hoseney, 2010; Hinton, 1958; Kim & Flores, 1999). The presence of bran in a flour causes discolouration and a decreased final product quality, as well as a decreased shelf life due to lipid oxidation and rancidity (Delcour & Hoseney, 2010; Doblado-Maldonado *et al*., 2012). According to South African wheat flour regulations, the ash content of white bread wheat flour must be between 0.60% and 1.0% (with a tolerance level of 0.05% from the maximum or minimum) (Department of Agriculture, Forestry and Fisheries, 2017). Whole wheat flour's ash content is usually approximately 1.6% (Marshall, 2010). The mean ash content of stone milled flour (1.31 ± 0.04%) was significantly higher ($P \leq 0.05$) than roller milled flour (0.47 ± 0.1%) (Table 3.6), indicating that the sieving of stone milled flour using a 212 µm sieve was not sufficient for screening out the bran from the stone milled flour. This proved true when the two wheat samples were factored in, as the ash content of the stone milled Cultivar flour sample (1.31 ± 0.03%) and the stone milled Blend flour sample (1.31 ± 0.05%) both exceeded the 1.0% maximum. The roller milled Blend flour (0.44 ± 0.11%) and the roller milled Cultivar flour (0.51 ± 0.11%) were lower than the 0.6% minimum.

The high presence of bran in the stone milled flour is due to the milling process. During stone milling, the entire wheat kernel is crushed and reduced in size, which may result in either coarse or fine bran particles (Gélinas *et al.*, 2004). The larger bran particles were efficiently separated from the flour during sieving; however, the finer bran particles were evenly distributed throughout the flour as they were the same size as the endosperm particles. A combination of roller and stone milling could possibly produce a flour with the correct ash content, as the stone mill cracks the wheat kernel open before the roller mill reduces the endosperm flour particle size (Doblado-Maldonado *et al.,* 2012; Posner & Hibbs, 2011). This could lead to a reduced bran content in the final product, but not compromise on producing a product that can still be called a stone milled white bread wheat flour.

Protein content

The protein content (%) significantly differed (P≤0.05) when comparing wheat samples, with the Cultivar flour having a higher mean (14.25 ± 1.53%) than the Blend flour (13.5 ± 1.09%) (Table 3.6). This was to be expected due to genetic variance between the two wheat samples, as well as different growing conditions and locations (Carson & Edwards, 2009).

The milling method had the largest effect on the flour samples, compared to the wheat sample and the wheat sample x milling method. The protein content of stone milled flour samples (14.89 ± 0.89%) were significantly higher (P≤0.05) than the roller milled flour samples (12.41 ± 0.4%). This may be because of the higher bran content of stone milled flour samples, as indicated by the higher ash content and darker colour than the roller milled samples. A higher protein content does not always indicate a better baking quality (or a higher loaf volume), as this can only be confirmed with a baking test (Gabriel *et al*., 2017; Gan *et al*., 1992). Wheat proteins consist mainly of gluten and non-gluten proteins. Gluten proteins make up 80-85% of the kernel's protein content and nongluten proteins 15-20%. Gluten proteins are found in the endosperm and differ in the functionality of the non-gluten proteins found in the outer layers (including the bran) of the wheat kernel (Delcour & Hoseney, 2010; Veraverbeke *et al*., 2010). The endosperm consists of a gluten proteins that form a protein matrix of starch granules. The gluten proteins, which are the storage mechanisms of wheat, form a protein matrix of starch granules. Gluten proteins consist of gliadin and glutenin and are essential for breadmaking as they form a visco-elastic dough once hydrated. Gliadin is responsible for the dough's viscosity and plasticity and glutenin for the dough's strength and elasticity. Nongluten proteins, which consist of albumins and globulins, play a role in structural and metabolic characteristics, and do not contribute to the development of a dough.

The wheat bran contains non-gluten proteins which contribute to the total protein content of the stone milled flour. The presence of nongluten proteins in stone milled flour due to the higher bran content may therefore result in a higher total protein content of the stone milled flour compared to the roller milled flour.

Colour

The colour of a white wheat flour plays an important role as the consumer expects it to be a bright, white colour (Heiniö *et al*., 2016). The CIELab colour scale consists of three colour parameters: the L* (brightness or lightness), the a*(green-red or redness value) and the b* (blue-yellow or yellowness value).

The whiteness of flour is mostly influenced by the brightness and the yellowness (Oliver *et al.*, 1993). The brightness (L*) can be attributed to the milling process, as this is influenced by the bran content and the flour particle size. The closer the brightness value is to 100, the whiter the flour. The roller milled flour (93.24 ± 0.23) was significantly whiter ($P \leq 0.05$) than the stone milled flour (87.49 ± 0.70) with regards to the mean L* value (Table 3.6). This is because the roller milled flour is more refined than the stone milled flour due to the lower bran content, which is of a darker brown colour than the whiter endosperm (Posner & Hibbs, 2011). As the particle size of white flour decreases, the surface area of the flour increases and allows more light to reflect off the flour. However, despite the stone milled flour having a smaller particle size than the roller milled (Table 3.6), the dark bran particles played a large role in the colour of the flour.

The higher mean yellowness (b*) values indicate that the stone mill flour (12.95 ± 0.35) is significantly more yellow ($P \leq 0.05$) than the roller milled flour (11.31 ± 0.31). The yellowness of the flour is mostly influenced by the carotenoid pigments found in the endosperm but can be destroyed by bleaching (Delcour & Hoseney, 2010; Oliver *et al*., 1993). Bleaching, however, does not affect the colour of the bran particles. The higher redness (a*) values of the stone milled flour (2.06 ± 0.08) indicate that they are significantly redder ($P \leq 0.05$) than the roller milled flour (0.68 ± 0.01).

The whiter colour of roller milled flour samples is acceptable, whereas the darker colour of the stone milled flour samples may negatively influence consumer acceptability of the product.

Particle size distribution

The median particle size of stone milled flour (71.56 ± 7.09 µm) was significantly smaller ($P \leq 0.05$) than roller milled flour (91.07 ± 1.71 µm) (Table 3.6). The Blend wheat roller milled flour (BR) produced the highest volume of larger flour particles, as indicated by the highest peak (Figure 3.1). The Blend wheat roller milled flour also had the largest median particle size (92.38 ± 0.68 µm), however this did not significantly differ from the median particle size of the Cultivar wheat roller milled flour (CR) at 89.75 ± 1.35 µm (Table 3.6). The Cultivar wheat stone milled flour (CS) and Blend wheat stone milled flour (BS) significantly differed ($P \leq 0.05$) from each other, as well as from the roller milled samples. The Cultivar stone milled samples (66.05 ± 2.02 µm) had the finest particle size, and the Blend stone milled samples (77.06 ± 5.83 µm) the second finest.

The smaller particle size of the stone milled flour may be due to the grinding severity of stone milling and indicates that the stone mill produces small amounts of a fine flour as the flour yield was much lower for stone than roller milled flour. This could be because the abrasiveness and aperture of the millstones were effective in reducing the particle size (Gèlinas *et al*., 2004). The smaller particles result in a higher water absorption due to the increased surface area of the particles, which affects the mixing and pasting properties of a flour (Posner & Hibbs, 2011).

The stone milled flour samples in this study had peaks at *ca*. 1 µm, 90 µm and 200 µm, with the highest volume recorded at the second peak (Figure 3.1). The findings in this study are not dissimilar to that of Cappelli *et al*. (2020), which was the only previous study that studies the particle size distribution of white stone milled flour. The particle size distribution of the white stone milled flour produced by Cappelli *et al*. (2020) had a trimodal curve with peaks at 0.1-1 µm, 20-30 µm and 200-300 µm, with only the second peak not being in the same range as this study. The roller milled flour samples had a very distinguishable peak at *ca*. 100-110 µm (Figure 3.1). This peak was also higher than the stone milled flour, which indicates that there were more flour particles of this size range.

Other studies presented contrasting results regarding particle size as they employed different stone mills and were on whole wheat stone milled flour. Ross & Kongraksawech (2018) indicated that the finest whole wheat flour was produced by a stone mill, however it did not significantly differ from that of a roller milled flour – the median sizes ranged between 108.3 and 111.5 µm. The particle size of the whole wheat stone milled flour investigated by Kihlberg *et al*. (2004) contrasted with Ross & Kongraksawech (2018), as the stone milled flour was medium sized, whereas the roller milled flour was either very fine or very coarse.

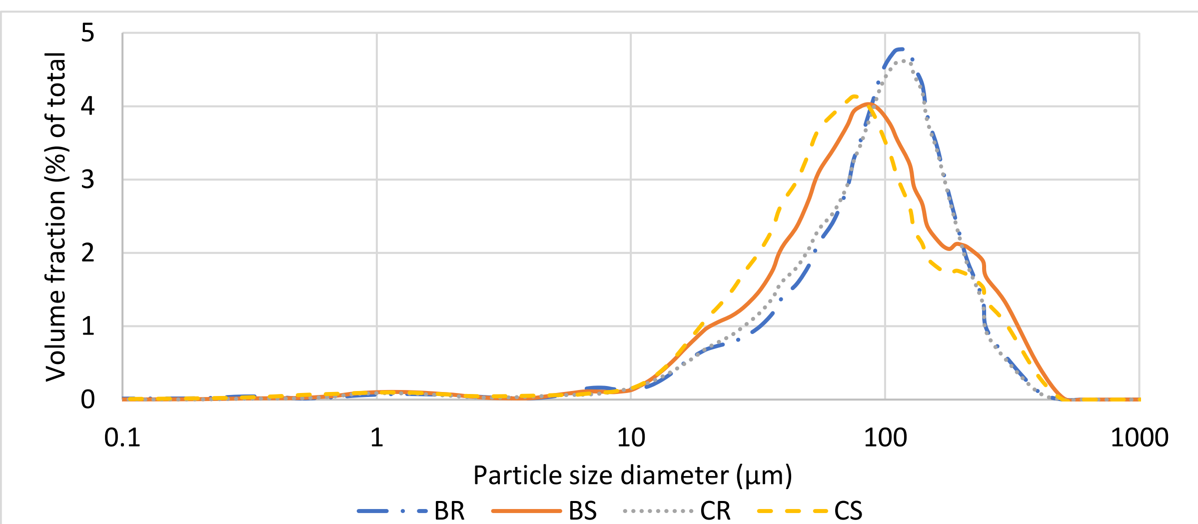

Figure 3.1 Particle size distribution of the Blend wheat roller milled flour (BR), Blend wheat stone milled flour (BS), Cultivar roller milled flour (CR) and a Cultivar wheat stone milled flour (CS) samples.

Table 3.7 The gluten analyses as well as the falling number (s) and starch damage (%) analyses of the flour samples

Mixed model ANOVA	Gluten analyses				Starch damage (%)	Falling number (s)
	Wet gluten content (%)	Gluten index	Dry gluten content (%)	Water binding capacity (%)		
F values (p values)						
Wheat sample	128.64 (≤0.05)	6.363 (≤0.05)	99.95 (≤0.05)	5.13 (≤0.05)	24.71 (≤0.05)	19.48 (≤0.05)
Milling method	131.43 (≤0.05)	0.01 (0.93)	103.01 (≤0.05)	4.41 (≤0.05)	455.86 (≤0.05)	12.21 (≤0.05)
Wheat sample x milling method	8.89 (≤0.05)	1.02 (0.32)	13.03 (≤0.05)	137.25 (≤0.05)	0.6 (0.45)	6.4 (≤0.05)
Mean: Wheat sample						
Cultivar	41.23 ± 4.41^{a}	94.9 ± 1.67^{b}	14.19 ± 1.49^{a}	32.79 ± 3.64^{b}	10.43 ± 2.08^{a}	394.42 ± 12.77^{a}
Blend	35.05 ± 2.6^{b}	96.79 ± 1.91^{a}	12.23 ± 0.79^{b}	33.85 ± 2.52^{a}	9.55 ± 1.95^{b}	378.42 ± 10.66^{b}
Mean: Milling method						
Roller mill	35.02 ± 2.51^{b}	95.88 ± 2.12^{a}	12.22 ± 0.68^{b}	33.81 ± 2.44^{a}	8.11 ± 0.42^{b}	380.08 ± 11.21^{b}
Stone mill	41.23 ± 4.41^{a}	95.81 ± 1.97^{a}	14.2 ± 1.52^{a}	32.83 ± 3.71^{b}	11.88 ± 0.77^{a}	392.75 ± 14.25^{a}
Mean: Wheat sample x milling method						
Cultivar x roller mill	37.28 ± 1.15^{b}	95.31 ± 2.11^{ab}	12.84 ± 0.14^{b}	36.01 ± 1.14^{a}	8.48 ± 0.12^{c}	383.5 ± 7.31^{b}
Cultivar x stone mill	45.11 ± 2.18^{a}	94.49 ± 1.11^{b}	15.53 ± 0.71^{a}	$29.58c \pm 1.76^{c}$	12.39 ± 0.53^{a}	405.33 ± 4.37^{a}
Blend x roller mill	32.75 ± 0.43^{c}	96.44 ± 2.16^{ab}	11.59 ± 0.24^{c}	31.61 ± 0.41^{b}	7.73 ± 0.21^{d}	376.67 ± 13.97^{b}
Blend x stone mill	37.35 ± 1.4^{b}	97.14 ± 1.76^{a}	12.87 ± 0.58^{b}	36.09 ± 1.35^{a}	11.37 ± 0.64^{b}	380.17 ± 6.91^{b}

Values are mean ± standard deviation of 3 batches in duplicate (wheat sample: n=12; milling method: n=12; wheat sample x milling method: n=6)
Mean values with different superscripts in a column differ significantly (P≤0.05)

Gluten analyses

The wet gluten content (%) and the dry gluten content (%) of stone milled flour was found to always be significantly higher (P≤0.05) than roller milled flour (Table 3.7). The wet gluten content of the stone milled flour was 41.23 ± 4.41% and the roller milled flour was significantly lower (P≤0.05) at 35.02 ± 2.51%. The dry gluten content of the stone milled flour was 14.2 ± 1.52% and the roller milled flour was 12.22 ± 0.68%.

The increased gluten contents of the stone milled flour may likely not be an accurate representation of the gluten content and quality due to the larger bran particles that are found in the stone milled flour. These bran particles were visible in the colour difference of the dry gluten (Figure 3.2), with the roller milled flour being much lighter in colour than the browner shade of the stone milled flour. The stone milled flour may produce a much weaker gluten network, mainly due to the disruption caused by the bran and germ particles, as well as bran competing with the gluten and starch for water absorption (Heiniö *et al*., 2016). The bran may also be responsible for gluten dilution in the dough (Hemdane *et al*., 2016). If the gluten network is compromised due to the bran content, the dough's resilience will decrease, thus negatively affecting the gas retention in the dough. This could lead to poor quality bread being baked that has a low loaf volume and a dense crumb (Gan *et al*., 1992; Li *et al*., 2012).

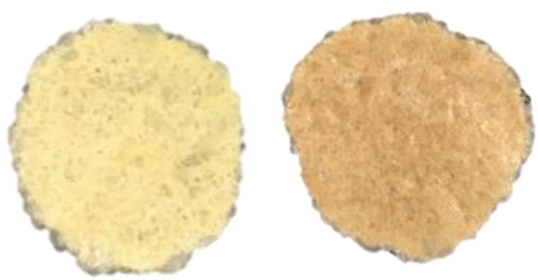

Figure 3.2 The dried gluten of the roller milled Cultivar flour (left) is much lighter in colour than the stone milled Cultivar flour (right).

The gluten index (GI), which is an indicator of the protein quality of the wheat and the strength of the gluten, was not significantly different (P=0.93) between the stone and roller milling methods (Table 3.7). The reason for there only being a significant difference between the wheat samples (Cultivar and Blend wheat) is that the gluten index is influenced by the wheat Cultivar and the environment the wheat is grown in (Bonfil & Posner, 2012). A GI close to zero indicates a very weak gluten, making the wheat suitable for animal feed. If the GI is between 55 and 100, as with all the flour samples in this study, it is suitable for breadmaking.

Starch damage

Starch is an essential food source of fermentable sugars that is utilised by yeast during fermentation (Carson & Edwards, 2009). Starch damage occurs when starch granules have been physically altered due to mechanical damage during milling, producing smaller, fractured starch particles (Ghodke *et al*., 2009). Starch can be damaged by either the scratching effect of the mill's grooves or furrows, or when the granules are physically reduced in size during the milling process. The stone milled flour (11.88 ± 0.77%) had a significantly higher (P≤0.05) mean starch damage than the roller milled flour (8.11 ± 0.42%), possibly due to the increased milling severity of the stone mill (Ross & Kongraksawech, 2018) (Table 3.7). The increased starch damage of the stone milled flour caused the water absorption to also be higher compared to the roller milled flour (Carson & Edwards, 2009). This is because the broken granules of damaged starch absorb water more easily.

A possible way to limit the amount of starch damage of stone milled flour is by increasing the feed rate of the wheat and decreasing the distance between stones (Ghodke *et al*., 2009). If starch damage is excessive, the granules may be more susceptible to enzymatic activity such as *alpha*-amylase hydrolysis (Pasha *et al.*, 2010). *Alpha*-amylase hydrolysis may lead to the dough being extremely sticky and difficult to work with, excessive proofing, a sticky crumb and redder crust colour (Carson & Edwards, 2009; Ghodke *et al*., 2009).

Falling number

The falling number (FN) is associated with the *alpha*-amylase activity in flour and is recorded in seconds (Table 3.7). The FN is inversely proportional to the *alpha*-amylase activity. If the FN is 65 s, which is the lowest possible measurement on the apparatus, the sample contains a high *alpha*-amylase activity (Posner & Hibbs, 2011). This could indicate a stickier dough due to increased complex sugar compounds; as well as a higher ash content, and a darker colour (Carson &Edwards, 2009; Lorenz & Valvano, 1981).

There was a significant difference (P≤0.05) between the FN of the wheat samples, milling methods and wheat sample x milling methods (Table 3.7), however all the flour samples were above 350 s. A FN value of approximately 300-350 s for hard wheat is desired as it indicates optimal baking quality and is often required by bread producers (Carson & Edwards, 2009; Posner & Hibbs, 2011). The FN of the Cultivar flour samples (394.42 ± 12.77 s) was significantly higher (P≤0.05) than the Blend flour samples (378.42 ± 10.66 s).

The means of the milling methods indicated that the stone milled samples (392.75 ± 14.25 s) had a significantly higher (P≤0.05) FN than the roller milled samples (380.08 ± 11.21 s), however both these values are well above the 300-350 s standard for optimal baking quality. The higher FN of the stone milled flour may be due to the increased levels of starch damage recorded in stone milled flour in this study (Posner & Hibbs, 2011).

3.3.5 Functional properties

The functional properties of the stone and roller milled flour samples, which include the consistograph, alveograph, mixograph (Table 3.8) and Rapid Visco Analyser (RVA) pasting properties (Table 3.9). The mixograms (Figure 3.3) and alveograms (Figure 3.4), as well as the RVA pasting curves (Figure 3.5) illustrate the differences in the results of the four flour samples.

Consistograph

The water absorption capacity (WAC, %) measurements shown in Table 3.8 presented a significant difference ($P \leq 0.05$) between the milling methods as well as the wheat sample x milling method treatments, however not between the mean wheat sample treatments ($P=0.45$). The milling methods showed the largest variance ($F=77.87$) compared to the wheat sample x milling method ($F=15.48$).

The WAC of the stone milled flour samples (63.42 ± 1.11%) was significantly higher ($P \leq 0.05$) than that of the roller milled flour samples (59.36 ± 1.15%). This means that the stone milled flour samples require more water to become fully hydrated. This is also true when the different wheat samples were factored in (wheat sample x milling method): the Cultivar stone milled flour (64.1 ± 1.01%) and the Blend stone milled flour (62.73 ± 0.8%) had the highest water absorption capacity, and the Blend roller milled flour (60.6 ± 0.42%) and Cultivar roller milled flour (58.53 ± 0.12%) had the lowest.

The higher WAC of stone milled flour means that more water will be required to hydrate fully to form a dough of a specified consistency than for the roller milled flour. The water absorption is important when it comes to the production of bread as it affects the dough yield and economical gain as more water is needed to produce a dough if the WAC is higher. The stone milled flour samples had a higher water absorption than the roller milled flour samples due to the higher starch damage, protein and ash content (and thus a higher arabinoxylan content) and smaller median particle size (Table 3.7). Increased damaged starch levels result in an increase water absorption capacity due to the starch granules being able to absorb more water than native starch granules (Carson & Edwards, 2009). Particle size distribution also affected the water absorption of the flour – the smaller the particles, the larger the surface area and water absorption (Posner & Hibbs, 2011; Sapirstein *et al*., 2018). The stone milled flour had a smaller median particle size, as well as a higher bran content (and therefore more non-starch polysaccharides) than the roller milled flour, which likely increased the water absorption (Heiniö *et al*., 2016). Bran competes with gluten and starch for water absorption, which results in a lower loaf volume. Other factors, such as wheat hardness, may also influence the WAC (Sapirstein *et al*., 2018), however the wheat hardness in this study was similar for both wheat samples.

Mixograph

The mixograph peak time and the peak height indicate when the dough is optimally mixed (Delcour & Hoseney, 2010) (Figure 3.3). The midline peak time is the duration until the gluten network of the dough is optimally developed and all protein and starch are fully hydrated. The resistance of the dough to mixing increases until this optimal peak time, where after the peak height decreases due to overmixing (Delcour & Hoseney, 2010; Goesaert *et al*., 2005). The peak time of the stone milled flour (3.15 ± 0.57 min) was found to be significantly longer ($P \leq 0.05$) than the roller milled flour (3.07 ± 0.57 min) due to the compromised gluten network and the increased starch damage and bran content of the stone milled flour (Table 3.8). The longer mixing time of stone milled flour may result in the dough having an increased temperature, resulting in a shorter proofing time and lower water absorption and thus a lower loaf volume. The stone milled flour samples also had a significantly higher ($P \leq 0.05$) peak height (57.74 ± 7.9 mm) than roller milled flour samples (56.26 ± 4.39 mm).

The effect of the wheat flour samples (Cultivar and Blend) on the flour samples was larger than the mean milling method or mean wheat sample x milling method. The Cultivar flour samples developed much quicker to an optimally mixed dough (2.49 ± 0.14 min) than the Blend wheat flour samples (2.74 ± 0.11 min). This may be due to the higher protein content of the Cultivar wheat sample, which is due to genetic variety and environments that the wheat is cultivated in (Preston *et al*., 2001; SAGL, 2019).

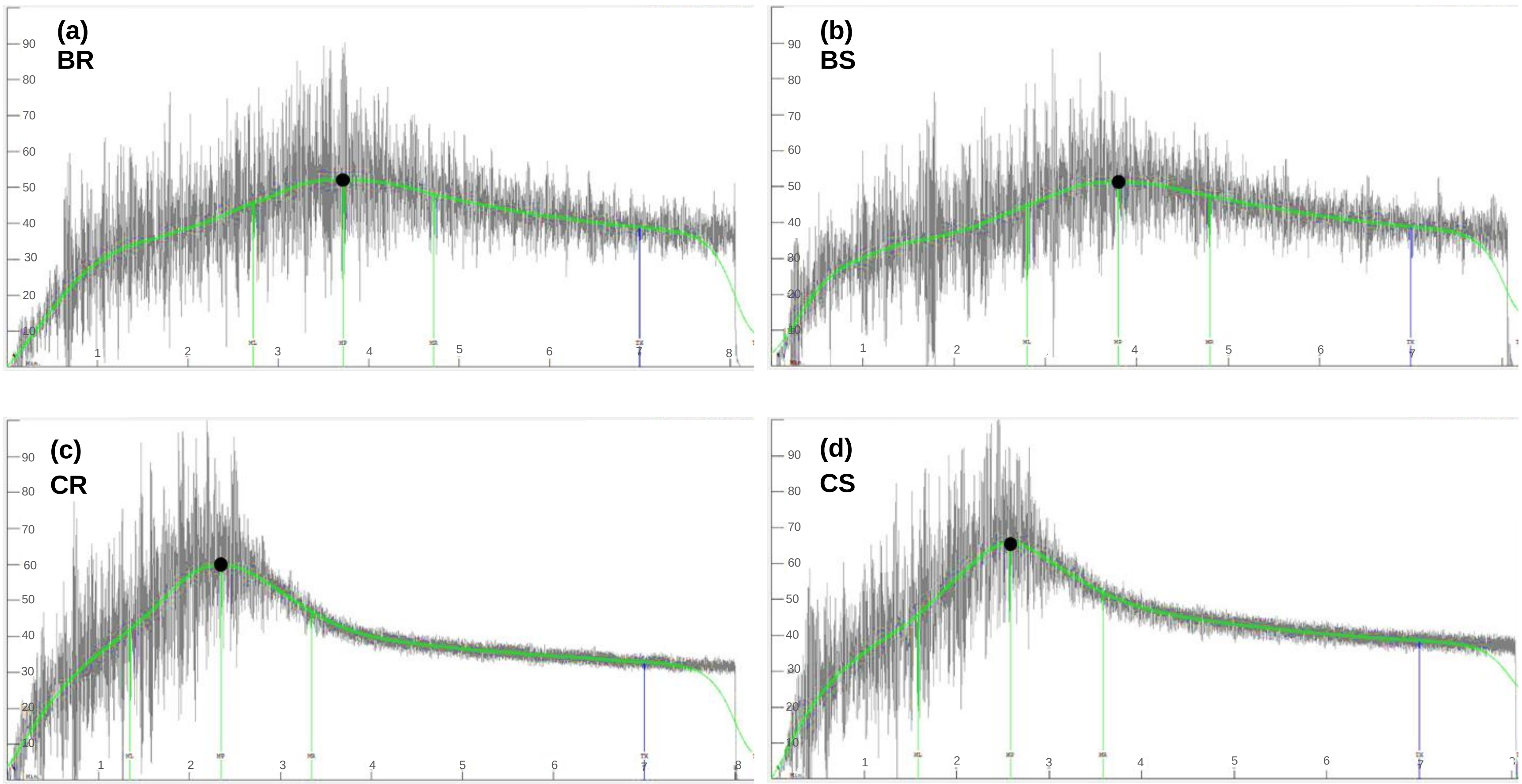

Figure 3.3 Mixograms of (a) Blend wheat roller milled flour (BR), (b) Blend wheat stone milled flour (BS), (c) Cultivar wheat roller milled flour (CR) and (d) Cultivar wheat stone milled flour (CS) samples. The solid black circle shows the midline peak height (y-axis, mm) and the midline peak time (x-axis, min).

Table 3.8 Functional properties of the flour samples as measured using an alveograph, consistograph and mixograph

Mixed model ANOVA	Consistograph	Mixograph		Alveograph			
	Water absorption capacity (%)	Midline peak time (min)	Midline peak height (mm)	P (mm)	L (mm)	P/L	W (10^{-4} J)
F values (p values)							
Wheat sample	0.64 (0.45)	1378.79 (≤0.05)	433.74 (≤0.05)	57.42 (≤0.05)	0 (0.99)	7.35 (≤0.05)	155.08 (≤0.05)
Milling method	77.87 (≤0.05)	5.8 (≤0.05)	7.14 (≤0.05)	219.99 (≤0.05)	9.88 (≤0.05)	201.55 (≤0.05)	20.39 (≤0.05)
Wheat sample x milling method	15.48 (≤0.05)	25.93 (≤0.05)	35.27 (≤0.05)	12.58 (≤0.05)	0.65 (0.43)	25.39 (≤0.05)	23.14 (≤0.05)
Mean: Wheat sample							
Cultivar	61.32 ± 3.12[a]	2.49 ± 0.14[b]	62.76 ± 2.85[a]	103.5 ± 12.41[b]	79 ± 18.82[a]	1.39 ± 0.41[b]	224.33 ± 20.76[b]
Blend	61.88 ± 1.32[a]	3.74 ± 0.11[a]	51.24 ± 1.51[b]	119.17 ± 20.92[a]	79.08 ± 35.44[a]	1.59 ± 0.77[a]	322.92 ± 42.66[a]
Mean: Milling method							
Roller mill	59.36 ± 1.15[b]	3.07 ± 0.75[b]	56.26 ± 4.39[b]	96 ± 5.03[b]	94.5 ± 32.09[a]	0.96 ± 0.15[b]	291.5 ± 74.08[a]
Stone mill	63.42 ± 1.11[a]	3.15 ± 0.57[a]	57.74 ± 7.9[a]	126.67 ± 13.68[a]	63.58 ± 7.69[b]	2.03 ± 0.37[a]	255 ± 36.98[b]
Mean: Wheat sample x milling method							
Cultivar x roller mill	58.53 ± 0.12[c]	2.36 ± 0.04[c]	60.38 ± 1.15[b]	91.83 ± 2.23[d]	90.5 ± 19.41[ab]	1.05 ± 0.18[c]	223.17 ± 27.45[c]
Cultivar x stone mill	64.1 ± 1.01[a]	2.62 ± 0.07[b]	65.14 ± 1.73[a]	115.17 ± 2.64[b]	67.5 ± 9.22[bc]	1.74 ± 0.25[b]	225.5 ± 13.81[c]
Blend x roller mill	60.6 ± 0.42[b]	3.78 ± 0.13[a]	52.14 ± 0.58[c]	100.17 ± 2.99[c]	98.5 ± 43.02a	0.87 ± 0.04[c]	359.83 ± 10.61[a]
Blend x stone mill	62.73 ± 0.8[a]	3.69 ± 0.07[a]	50.33 ± 1.64[d]	138.17 ± 9.35[a]	59.67 ± 2.88[c]	2.32 ± 0.2[a]	286 ± 24.93[b]

Values are mean ± standard deviation of 3 batches in duplicate (wheat sample: n=12; milling method: n=12; wheat sample x milling method: n=6)
Mean values with different superscripts in a column differ significantly (P≤0.05)
P = resistance to extension or tenacity; L = extensibility; P/L = curve configuration ratio; W = deformation energy

Alveograph

The resistance of the dough to extension, also known as the tenacity, is indicated by the P-value (mm) (Table 3.8). The P-value is directly proportional to the strength of the dough. A higher P-value is often associated with a stronger dough. An acceptable P-value is in the range of 65-120 mm and is indicated by the y-axis of an alveogram (Figure 3.4) (SAGL, 2019). The milling method (stone and roller milling) had the largest influence on the P-value (with an F value of 219.99) (Table 3.8). The roller milled flour had a significantly lower (P≤0.05) P-value (96 ± 5.03 mm) compared to the stone milled flour (126.67 ± 13.68 mm). The roller milled Cultivar wheat (91.83 ± 2.23 mm), stone milled Cultivar (115.17 ± 2.64 mm) and the roller milled Blend (100.17 ± 2.99 mm) flour samples all fall within the acceptable P-value range. The Blend wheat stone milled flour (138.17 ± 9.35 mm) was the only sample exceeding 120 mm. The highest P-value was recorded with the Blend wheat flour that was stone milled (138.17 ± 9.35 mm) and the lowest with the Cultivar wheat flour that was roller milled (91.83 ± 2.23 mm). This indicates that the stone milled flour could produce a stronger dough compared to roller milled flour.

The L-value (x-axis, mm) represents the extensibility of the dough and is found acceptable when between 80 and 120 mm (SAGL, 2019). The roller milled flour and the stone milled flour showed a significant difference (P≤0.05) in the L-value, with the roller mill (94.5 ± 32.09 mm) falling in the acceptable range and being significantly higher (P≤0.05) than the stone mill (63.58 ± 7.69 mm), indicating that the roller milled flour was more extensible. The mean wheat sample treatment (P=0.99) and the mean milling method x wheat sample treatment (P=0.43) did not show a significant difference. However, the roller milled flour for both the Blend (98.5 ± 43.02 mm) and the Cultivar (90.5 ± 19.41 mm) had the highest L-values and were within the acceptable range of 80-120 mm. The L-values of the stone milled flour samples were much lower and below the acceptable levels: the mean L-value of the Cultivar flour samples was 67.5 ± 9.22 mm and the Blend wheat flour samples was the lowest at 59.67 ± 2.88 mm. This could indicate that the stone milled flour will produce a bread that has a very low loaf volume as the gluten is not elastic enough (Veraverbeke & Delcour, 2010).

The P/L-value is the curve configuration ratio. There was a significant difference (P≤0.05) between the mean P/L-value of the milling methods as well as the interaction between the wheat sample x milling method. A P/L-value that is close to 1 suggests a good relationship between the tenacity (P-value) and the extensibility (L-value), however a value of 0.70-1.50 is acceptable in the industry (SAGL, 2019). The P/L value of the roller milled flour samples were closest to 1, with the stone milled samples approximately double that. The mean P/L of the stone milled flour was 2.03 ± 0.37, with the roller milled flour having a

P/L of 0.96 ± 0.15. A similar inclination was observed when the wheat sample was also factored in. The stone milled Blend flour (2.32 ± 0.2) and the stone milled Cultivar flour (1.74 ± 0.25) both had very high P/L-values, which was due to the low L-value. This indicates that the dough of the stone milled flour was less extensible than the roller milled flour. The values of the stone milled flour falls outside the acceptable limits of 0.70-1.50 and could suggest a low loaf volume (SAGL, 2019). Both of the roller milled flour samples fall within these limits: the Cultivar wheat flour's value was 1.05 ± 0.18 and the Blend wheat flour was 0.87 ± 0.04.

The W-value is the deformation energy (the energy required to inflate the dough bubble until it ruptures) and is indicated by the area under the alveograph. It indicates the strength of the flour and therefore the strength of the dough. The optimal deformation energy depends on the final product and is closely related to the P/L value. There was once again a significant difference ($P \leq 0.05$) between the milling methods, as well as the wheat samples and the wheat sample x milling method. The stone milled samples showed a significantly lower ($P \leq 0.05$) deformation energy ($255 \pm 36.98 \times 10^{-4}$ J) than the roller milled samples ($291.5 \pm 74.08 \times 10^{-4}$ J). The roller milled Blend wheat flour was the only sample that had a W-value higher than 300×10^{-4} J, indicating a strong flour that could produce a good quality loaf (SAGL, 2019).

Starch damage and particle size influences the water absorption of a flour and may cause an increase in the P and P/L-values and a decrease in the L-value of hard wheat flour (Preston *et al.*, 1987). Stone milled flour has been characterised with more starch damage and higher ash content, as well as a smaller particle size, than the roller milled flour, which could explain the high P/L value. The increased starch damage and bran content results in a higher water absorption, and subsequently a stiffer dough and inhibited gluten formation (Dexter *et al.*, 1994; Li *et al.,* 2014). The bran in the stone milled flour may also cause the bubbles to burst prematurely due to damage to the gluten matrix (Li *et al.,* 2014), which may be the reason for the lower extensibility of the stone milled flour. Usually, an increase in the protein content causes an increase in the extensibility (Dexter *et al.*, 1994), however the protein constituents and functionality between the roller and stone milled flours could have differed due to the stone milled flour having more bran than the roller milled flour. The protein content of the stone milled flour was higher than the roller milled flour (Table 3.6), but there is a possibility that the (non-gluten) protein content does not contribute to the visco-elasticity of the dough, as measured by the alveograph.

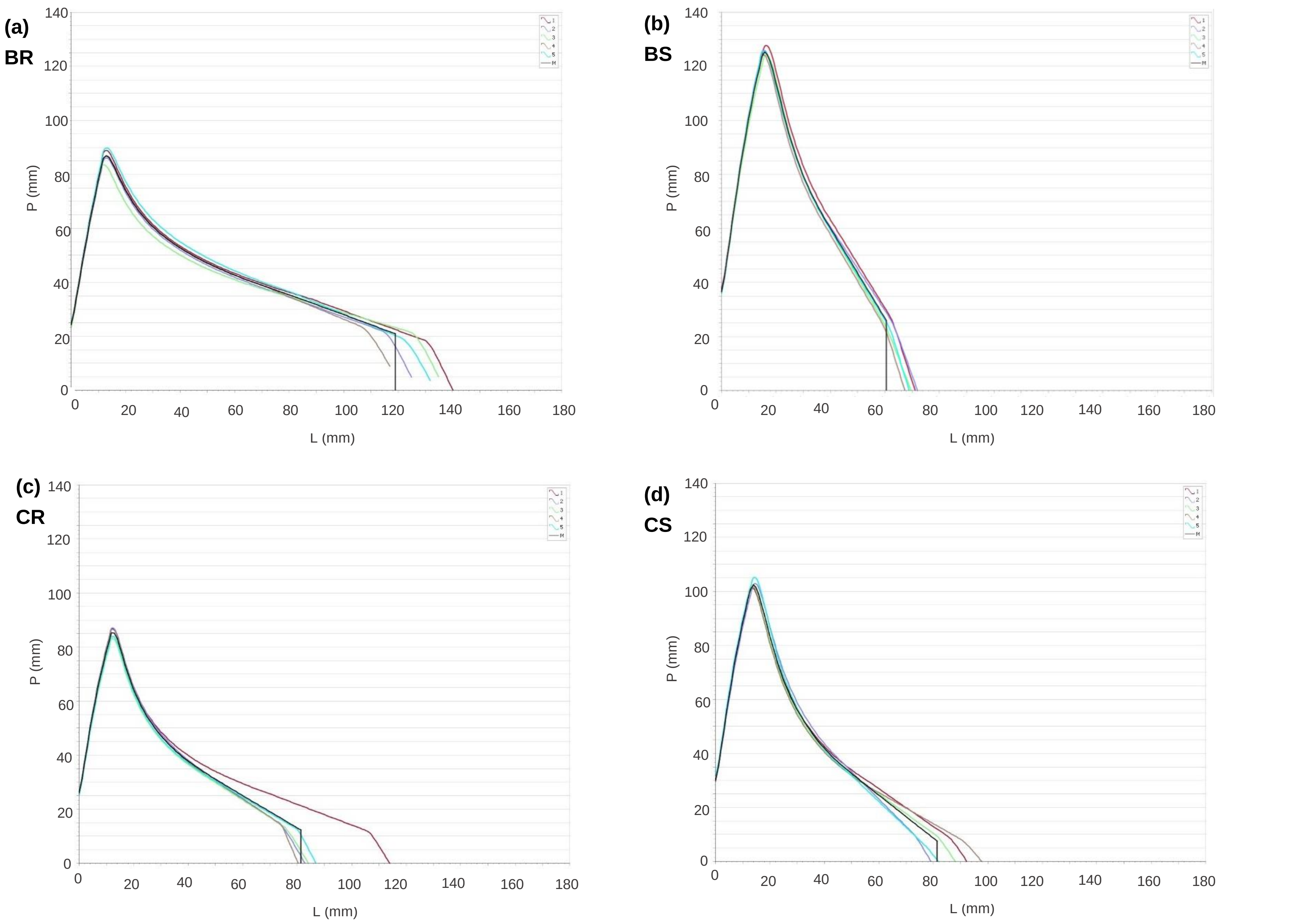

Figure 3.4 Alveograms of (a) blend wheat roller milled flour (BR), (b) blend wheat stone milled flour (BS), (c) cultivar wheat roller milled flour (CR) and (d) cultivar wheat stone milled flour (CS) samples.

Pasting properties

During the Rapid Visco Analyser (RVA) standard profile 1 test, there are five stages: initial, heating, holding, cooling and final holding stages (Balet *et al*., 2019). The variance in the temperature and time of each of these stages influences the starch-water slurry, thus allowing different pasting properties to be measured. The pasting properties of the flour samples, as determined by the RVA, are presented in Table 3.9. Viscosity occurs as a result of the temperature-dependent pasting when water is absorbed and plays a large role in the final product's quality (Barrera *et al*., 2013a).

The peak time is where the maximum viscosity occurs (Ragaee & Abdel-Aal, 2006). The pasting temperature is the temperature where pasting commences and viscosity increases. The peak time and pasting temperature are related to the rate that the starch granules absorb water and swell until the peak viscosity (Vp) is reached. Both the peak time and pasting temperature were influenced significantly by the milling methods ($P \leq 0.05$). The roller milled flour had a lower pasting temperature and longer peak time than the stone milled flour (Table 3.9).

All the pasting viscosities (Vp, Vb, Vf, Vt and Vs) were significantly influenced by the milling method ($P \leq 0.05$), which also had the largest effect size (F-values) when compared to wheat samples and wheat samples x milling methods. These pasting viscosities were always significantly lower for the stone milled flour samples than the roller milled flour samples, as can be seen in Figure 3.5. A similar trend was found in a study on whole wheat stone milled flour regarding the pasting properties (Liu *et al*., 2015). The figures of the stone milled samples (of both the Cultivar and the Blend flours) illustrate lower peaks and troughs than the roller milled samples. The lower viscosity of the stone milled flour compared to the roller milled flour may be due to the higher damaged starch content, smaller flour particles and higher water absorption capacity of stone milled flour. Damaged starch granules dissolve much quicker than whole starch granules, thus the lower viscosity values (Barrera *et al*., 2013a).

The peak viscosity (Vp) is the highest viscosity recorded at the end of the heating stage and results in the pasting of the starch granules (Balet *et al*., 2019; Ragaee & Abdel-Aal, 2006). It plays a large role in the quality of the final product, specifically the firmness of the bread during storage. The Vp, which was mostly influenced by the milling method, was also significantly affected ($P \leq 0.05$) by the wheat sample, however not by wheat sample x milling method ($P = 0.99$) (Table 3.9). The water absorption capacity (WAC) influences the degree that the starch granules swell during this heating stage, and in turn also influences the Vp (Balet *et al*., 2019; Ragaee & Abdel-Aal, 2006). As the stone milled flour in this study had a higher

WAC due the increased starch damage, protein and bran content, the degree of swelling was much higher, thus resulting in a lower Vp (1532.5 ± 207.06 cP) than the roller milled flour (2351.33 ± 230.63 cP).

The trough viscosity, or the holding strength, measures the lowest viscosity during the test (Ragaee & Abdel-Aal, 2006), and was lowest for the stone mill (988.08 ± 34.57 cP) compared to the roller mill (1659.5 ± 98.66 cP).

The breakdown viscosity (Vb) (which is the trough viscosity (Vt) subtracted from the peak viscosity) of the stone mill (544.42 ± 180.2 cP) was significantly lower ($P \leq 0.05$) than the roller milled flour (691.83 ± 176.88 cP). The breakdown viscosity is recorded during the process of the starch granules disintegrating (Balet *et al*., 2019).

The setback viscosity (Vs) is measured during starch retrogradation and is the Vt subtracted from Vf (Balet *et al.*, 2019). It indicates the increase in the viscosity as the starch granules undergo cooling. Vs shows a significant difference when comparing wheat samples, as well as milling methods. The roller milled flour samples had the highest Vs (1052.08 ± 192.57 cP) and the stone milled flour samples (805.75 ± 52.54 cP) the lowest Vs. The stone milled flour therefore had the lowest rate of starch retrogradation and syneresis (Ragaee & Abdel-Aal, 2006). During the setback region starch molecules recombine to form a gel structure, thereby increasing the Vf.

The final viscosity (Vf) is measured at the end of the test and occurs after the cooling stage and during the final holding stage (Ragaee & Abdel-Aal, 2006). Like the other pasting properties, it was also mostly influenced by the milling method, indicating a significant difference between the stone and the roller milled flour ($P \leq 0.05$). The wheat sample and the wheat sample x milling method did not have a significant difference ($P=0.24$). Regarding the milling method, the final viscosity of the roller milled flour samples (2711.58 ± 140.98 cP) was much higher than that of the stone milled flour samples (1797.17 ± 48.63 cP).

Table 3.9 The pasting properties of the flour samples as measured by the Rapid Visco Analyser

Mixed model ANOVA	Pasting temperature (°C)	Peak time (min)	Vp (cP)	Vb (cP)	Vf (cP)	Vt (cP)	Vs (cP)
F values (p values)							
Wheat sample	3.46 (0.08)	7.96 (≤0.05)	150.43 (≤0.05)	78.78 (≤0.05)	4.76 (≤0.05)	16.57 (≤0.05)	19.46 (≤0.05)
Milling method	147.30 (≤0.05)	25.43 (≤0.05)	651.94 (≤0.05)	18.74 (≤0.05)	553.93 (≤0.05)	900.46 (≤0.05)	36.78 (≤0.05)
Wheat sample x milling method	0.87 (0.36)	0.79 (0.39)	0 (0.99)	1.47 (0.24)	1.49 (0.24)	3.45 (0.08)	4.45 (≤0.05)
Mean: Wheat sample							
Cultivar	79.21 ± 10.17[a]	6.08 ± 0.25[b]	1745.25 ± 433.87[b]	467 ± 148.3[b]	2296.75 ± 511.42[a]	1278.25 ± 331.5[b]	1018.5 ± 218.3[a]
Blend	76.45 ± 9.96[a]	6.26 ± 0.2[a]	2138.58 ± 434.91[a]	769.25 ± 61.55[a]	2212 ± 461.36[b]	1369.33 ± 377.36[a]	839.33 ± 88.18[b]
Mean: Milling method							
Roller mill	68.81 ± 1.08[b]	6.33 ± 0.24[a]	2351.33 ± 230.63[a]	691.83 ± 176.88[a]	2711.58 ± 140.98[a]	1659.5 ± 98.66[a]	1052.08 ± 192.57[a]
Stone mill	86.85 ± 5.3[a]	6.01 ± 0.08[b]	1532.5 ± 207.06[b]	544.42 ± 180.2[b]	1797.17 ± 48.63[b]	988.08 ± 34.57[b]	805.75 ± 52.54[b]
Mean: Wheat sample x milling method							
Cultivar x roller mill	69.5 ± 1.1[b]	6.21 ± 0.3[b]	2154.5 ± 109.49[b]	561.33 ± 164.11[c]	2777.67 ± 141.58[a]	1593.17 ± 59.28[b]	1184.5 ± 196.31[a]
Cultivar x stone mill	88.93 ± 0.33[a]	5.95 ± 0.06[c]	1336 ± 13.62[d]	372.67 ± 9.65[d]	1815.83 ± 17.13[c]	963.33 ± 15.33[c]	852.5 ± 13[bc]
Blend x roller mill	68.13 ± 0.47[b]	6.45 ± 0.04[a]	2548.17 ± 109.76[a]	822.33 ± 31.95[a]	2645.5 ± 114.88[b]	1725.83 ± 85.69[a]	919.67 ± 30.98[b]
Blend x stone mill	84.78 ± 7.17[a]	6.07 ± 0.04[bc]	1729 ± 38.26[c]	716.17 ± 23.45[b]	1778.5 ± 63.81[c]	1012.83 ± 30.4[c]	759 ± 25.68[c]

Values are mean ± standard deviation of 3 batches in duplicate (wheat sample: n=12; milling method: n=12; wheat sample x milling method: n=6)
Mean values with different superscripts in a column differ significantly (P≤0.05)
Vp = peak viscosity; Vb = breakdown viscosity; Vf = final viscosity; Vt = trough viscosity; Vs = setback viscosity

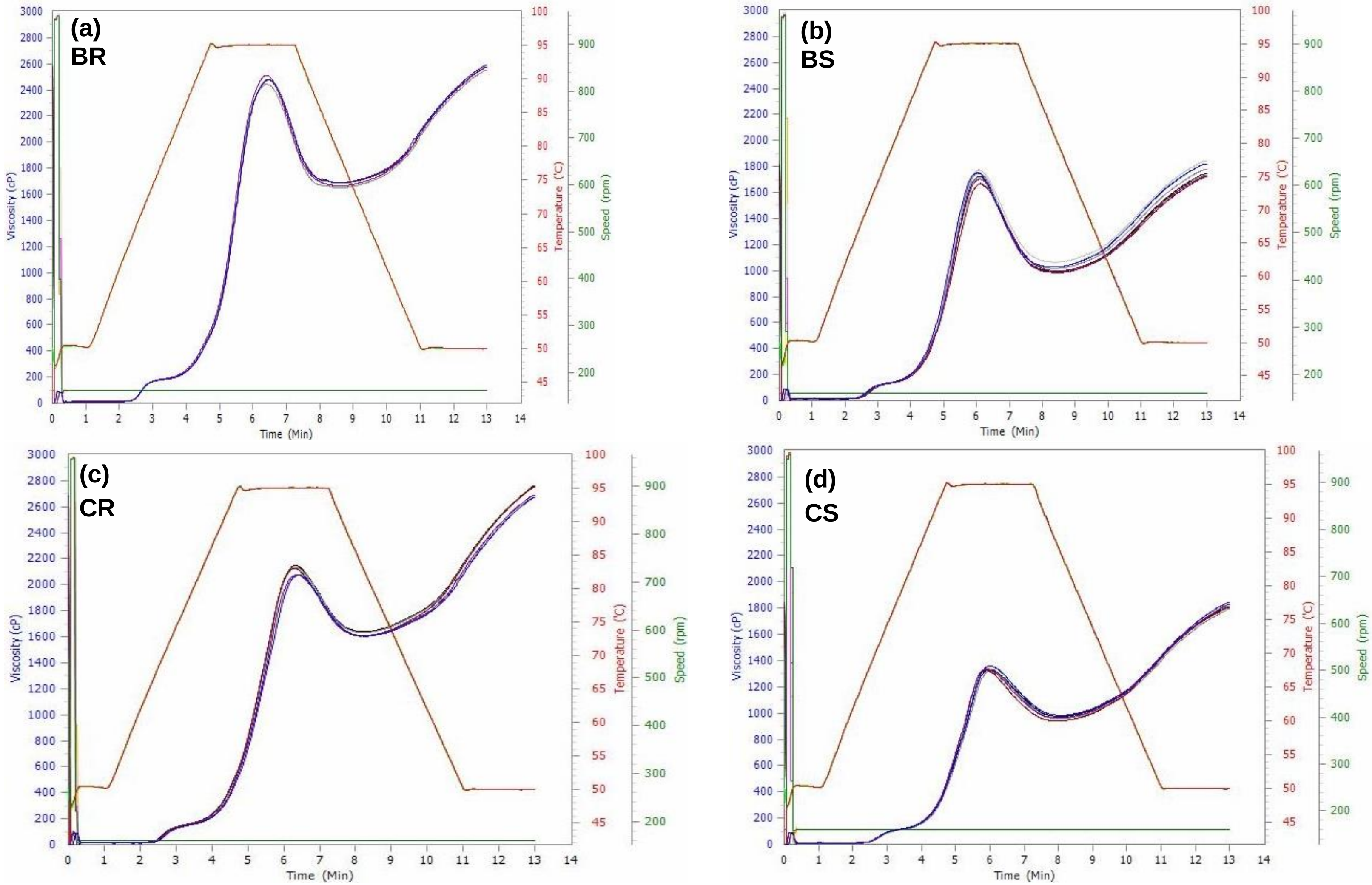

Figure 3.5 RVA pasting curves of the (a) Blend wheat roller milled flour (BR), (b) Blend wheat stone milled flour (BS), (c) Cultivar wheat roller milled flour (CR) and (d) a Cultivar wheat stone milled flour (CS) samples.

3.3.6 Principal component analysis (PCA)

The PCA biplot (Figure 3.6) accounts for 85.42% of the variance between the four combinations of wheat sample and milling method, namely Blend wheat roller milled flour (BR), Blend wheat stone milled flour (BS), Cultivar wheat roller milled flour (CR) and Cultivar wheat stone milled flour (CS) samples. These four flour samples formed four very distinct groupings of their three batches (eg. BR1, BR2, BR3), each in their own quadrant. BR is associated with the properties in the upper left quadrant, BS the upper right, BR the lower left and CS with the lower right.

The milling methods, namely stone and roller milling, indicated a large difference of 62.46% on PC1 (Principal Component 1, x-axis), which was much larger than PC2 (Principal Component 2, y-axis) of 22.96%. Stone milled flour was positively associated with properties in the right upper and lower quadrants of the PCA biplot. These properties included moisture content (%), P/L, water absorption capacity (WAC, %), ash content (%), starch damage (%), protein content (%), wet gluten (%) and dry gluten content (%). Roller milled flour, located in the left upper and lower quadrants of the PCA, were associated with properties such as the median particle size, L* (brightness/whiteness) and most of the pasting properties (Vb, Vp, Vt, Vf, Vs and peak time).

PC2 distinguished between the wheat samples, with Blend wheat samples in the upper quadrants and Cultivar flour samples in the lower quadrants. Properties that are influenced by wheat cultivars and genetic factors, such as gluten index and the mixograph's midline peak height and time, are found to be strongly associated with these variances.

The strong positive correlation between the properties of the stone milled flour, namely the high ash content, protein content, starch damage and WAC, was indicated by how closely they are situated to each other on the PCA biplot. The WAC is one of the properties positively affected by the starch damage: the more damaged the starch, the easier it is for the starch particles to absorb water. Alternatively, the ash content is angled nearly 180° from the L* colour value, indicating a strong negative correlation. The higher the ash content of the flour (as affected by an increase in bran particles) the lower the L* (or brightness) of the flour, due to the darker colour of the bran. There is also a strong negative correlation between the median particle size of roller milled flour and the WAC and starch damage. This was to be expected as the stone milled flour samples were finer (smaller median particle size) than the roller milled flour samples, and this affected the water absorption and starch damage. In a previous study (Ross & Kongraksawech, 2018), the high grinding severity of stone mills was associated with a high starch damage and finer particle size, and this was reflected in this study.

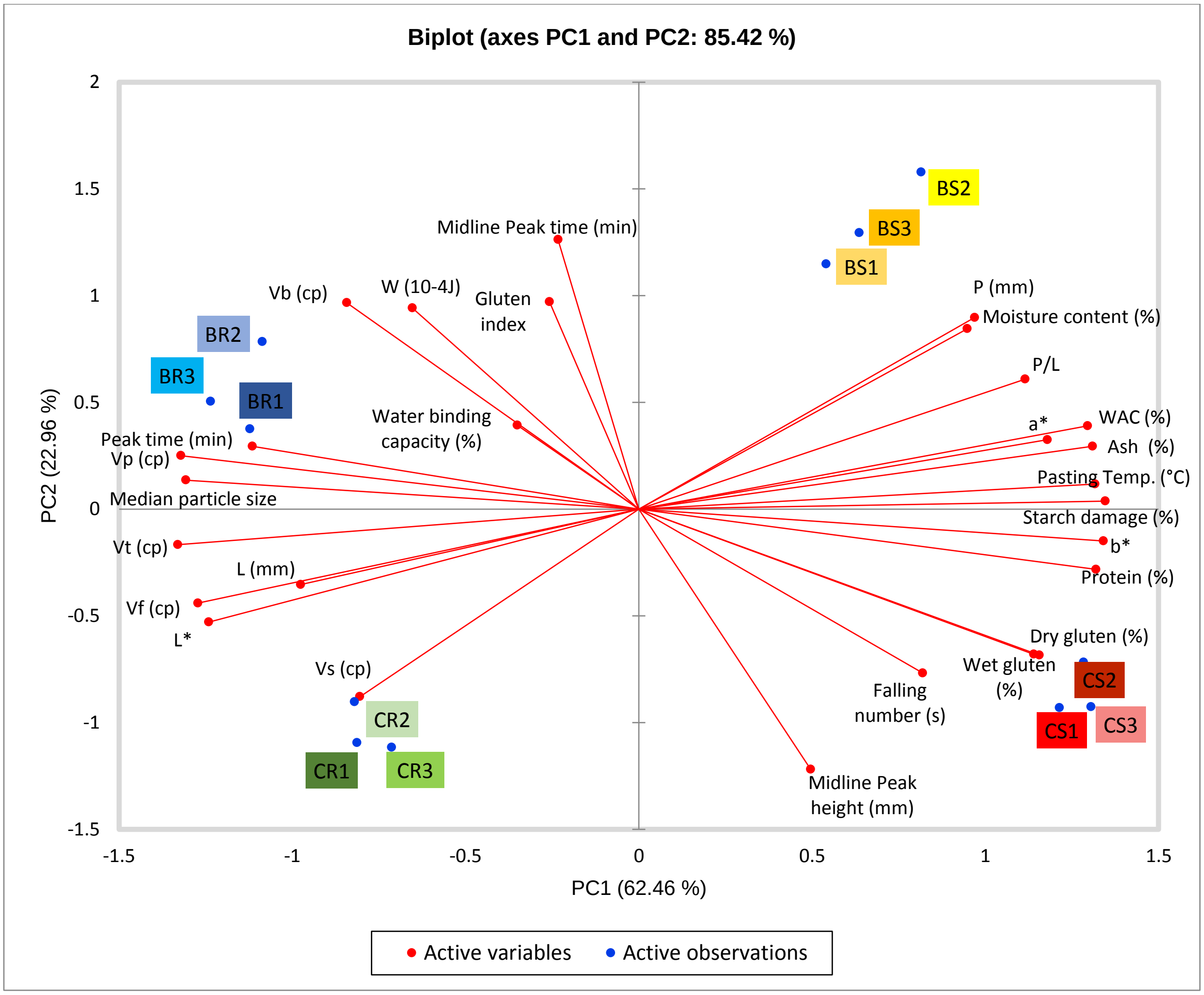

Figure 3.6 Principal component analysis (PCA) biplot illustrating the association between the physicochemical and functional properties for the four flour samples, namely Blend wheat roller milled flour (BR), Blend wheat stone milled flour (BS), Cultivar roller milled flour (CR) and Cultivar wheat stone milled flour (CS), with numbers 1-3 indicating the batch number.

3.3.7 Structural properties

Scanning electron microscopy

The scanning electron microscopy (SEM) micrographs of the flour samples provided a qualitative insight into the differences between the microstructural composition of the four flour samples, namely the Blend wheat roller, Blend wheat stone, Cultivar wheat roller and Cultivar wheat stone milled flours. Stone milled flour particles were more inhomogeneous compared to roller milled flour (Figure 3.7). The stone milled flour particles were also more irregularly sized with large groupings of protein-starch flour granules and bran particles clearly discernible, whereas the roller milled flour was more refined, consisting mostly of smaller protein-starch flour particles.

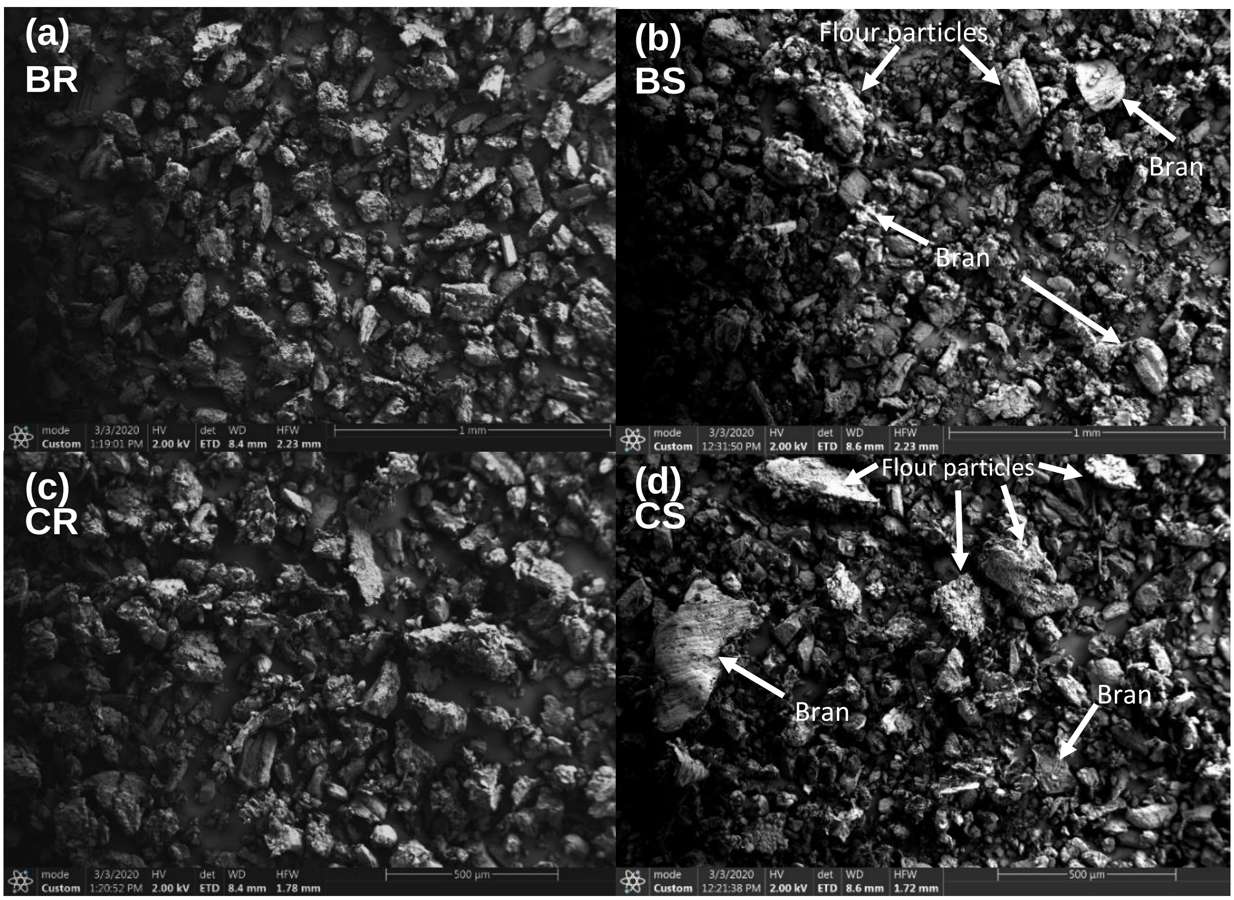

Figure 3.7 Scanning electron micrographs of (a) blend wheat roller (BR), (b) blend wheat stone (BS), (c) cultivar wheat roller (CR) and (d) cultivar wheat stone (CS) milled flour illustrating the more uniform roller milled flour particles and the larger and more irregular flour and bran particles in stone milled flour.

The bran particles in the stone milled flour samples (Figure 3.7b and Figure 3.7d) are more frequently visible and often much larger than other flour particles. An enlarged view of a fragmented and non-uniform bran particle found in a stone milled flour is illustrated in Figure 3.8. This bran particle has an uneven surface and a very clear, jagged edge where it was crushed by the stone mill.

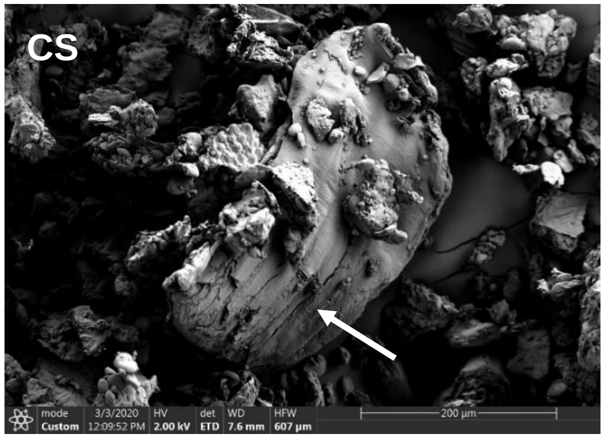

Figure 3.8 Scanning electron micrograph of a bran particle of a hard cultivar wheat flour that was stone milled (CS).

Figure 3.9a shows a grouping of starch granules that has a smooth surface except for the slight indentions from where other starch granules had pressed against it before physical separation. An example of the two types of starch granules are illustrated in Figure 3.9b. Type A starch granules are larger and have a lenticular shape and type B have a smaller, spherical shape (Delcour & Hoseney, 2010).

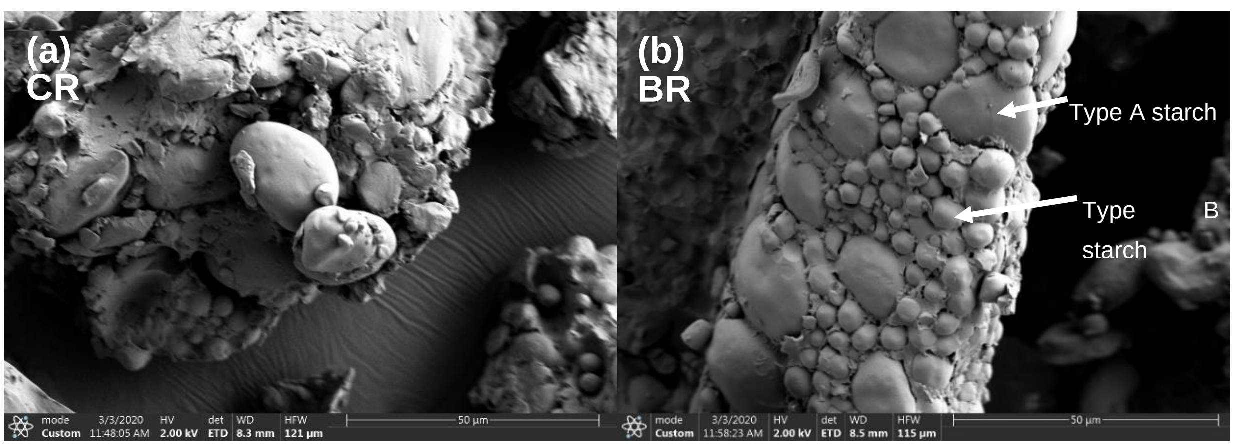

Figure 3.9 Scanning electron micrographs of starch granules of (a) cultivar wheat roller (CR) and (b) blend wheat roller (BR) milled flour which illustrates type A and type B starch granules.

Damaged starch, which has been strongly associated with stone milled flour in this study (Table 3.7) as well as others (Ross & Kongraksawech, 2018), is identified in Figure 3.10. The grinding severity of the stone mill leads to the starch granules undergoing more mechanical damage, thus more damaged starch. Damaged starch granules can be characterised by being a deformed shape, split, cracked and/or with an irregular surface (Barrera *et al*., 2013b). The starch granules of stone milled flour (Figure 3.10a-c) clearly have a more textured surface appearance than the roller milled flour (Figure 3.9). Figure 3.10a and Figure 3.10b also shows damaged starch granules that are noticeably deformed by being fragmented and irregularly shaped. However, not all the starch of the stone milled flours were damaged: Figure 3.11c clearly depicts a grouping of whole starch granules held together by a protein matrix.

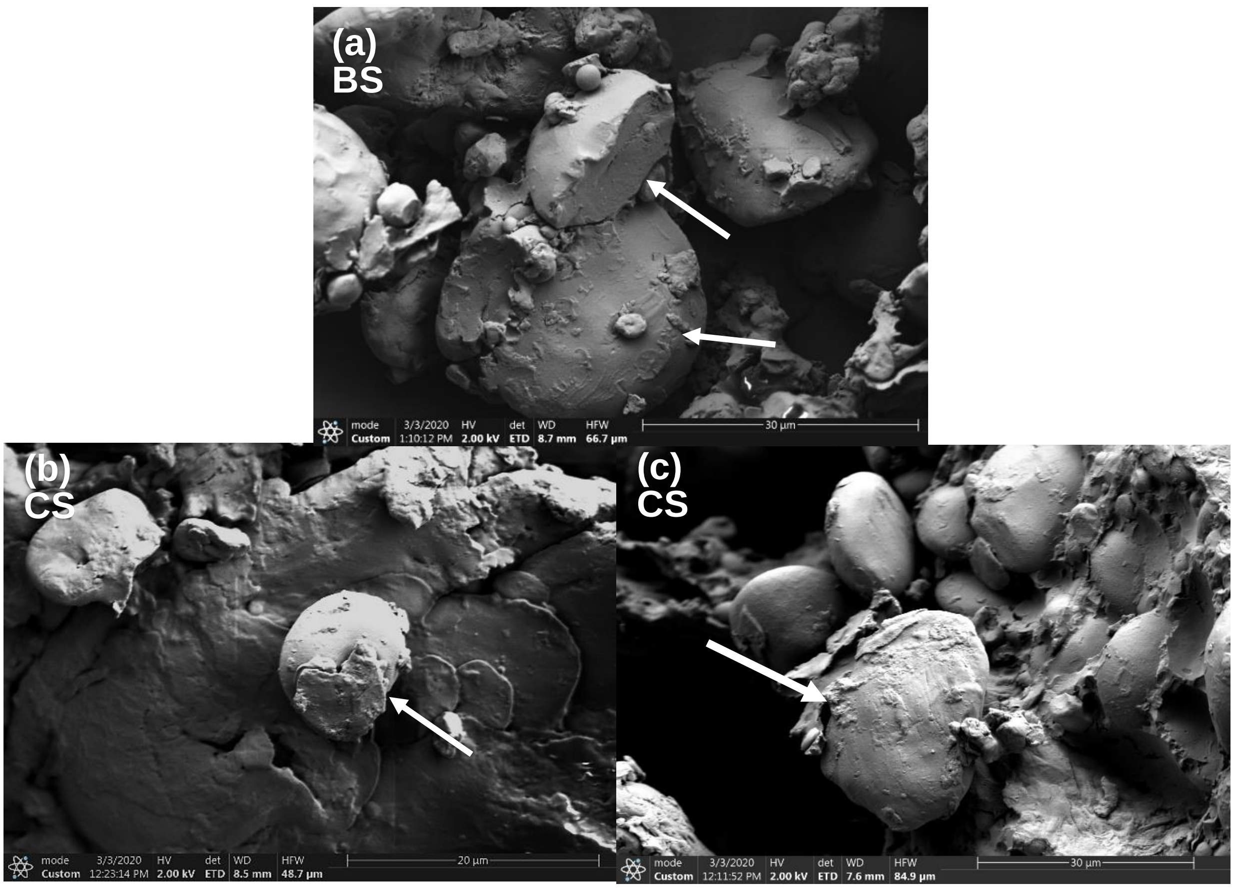

Figure 3.10 Scanning electron micrographs of stone milled wheat flours depicting damaged starch granules found in (a) blend wheat and (b) and (c) cultivar wheat flours.

The protein matrix, a thin membrane-like layer in which the starch granules are embedded, tended to be more intact and undisturbed in the roller milled flour (Figure 3.11a and Figure 3.11c) than the stone milled flour (Figure 3.11d). The starch granules in roller milled flour samples have not undergone much deformation and damage, due to less severe grinding and mechanical damage compared to the stone mill, thus also keeping the protein matrix intact. However, it should be noted that stone milled flour still had protein matrixes and starch granules that were intact (Figure 3.11c).

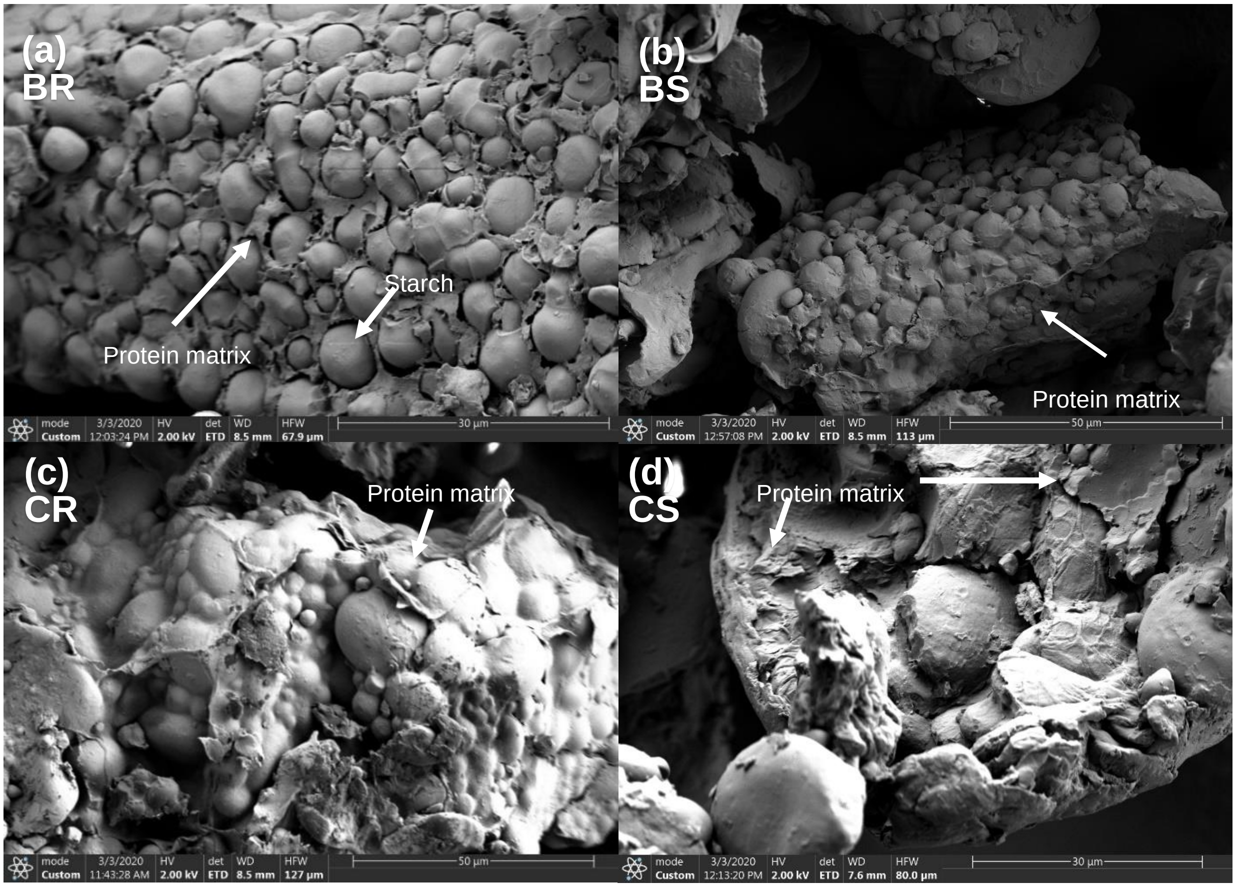

Figure 3.11 Scanning electron micrographs illustrating the membrane-like protein matrix of (a) blend wheat roller (BR), (b) blend wheat stone (BS), (c) cultivar roller (CR) and (d) cultivar wheat stone (CS) milled flours.

3.4 Conclusions

This research aimed to establish physicochemical, functional and structural properties of white stone milled flour and white roller milled flour, however it was not possible to produce a white stone milled flour using a commercial stone mill and sieving the whole wheat flour through a 212 µm laboratory sieve. Based on the South African wheat flour regulations, the ash content of the resulting sifted flour was too high to be classified as a white bread wheat flour. This may be due to dispersed bran particles, as the stone mill crushes the entire wheat kernel to a flour without first separating the bran and endosperm as with roller milling.

Limited work has addressed white stone milled wheat flour, and this research starts to fill the knowledge gap on how the physicochemical, functional and structural properties compare to those of roller milled flour. The stone milling process in this study resulted in a low refined flour yield (26.29-28-39%) compared to the roller milled flour (64.56-65.03%). The milling method (i.e. stone and roller milling) often had a larger effect on the properties of the flour samples than the different wheat types (i.e. Cultivar and Blend wheat) or the interaction of the milling method and wheat sample. Stone milled flour had a higher starch damage content than the roller milled flour, which was due to the grinding severity of the stones. Scanning electron micrographs illustrated the inhomogeneity of the stone milled flour. The micrographs also provided insight on the physical damage caused by the increased grinding severity of the stone mills on the damaged starch. The significantly darker colour and higher ash content of stone milled flour indicated a higher bran content. This is due to the stone milling process crushing the entire wheat kernel to a flour, whereas roller milling first removes the bran before flour particle size reduction takes place.

Aside from the higher bran content of stone milled flour, the higher levels of starch damage and protein content, as well as a smaller particle size resulted in a higher water absorption capacity. The stone milled flour samples absorbed more water, which caused the gluten network to be inhibited and the dough to be stiffer. Subsequently, this resulted in a higher alveograph P (tenacity of the dough) and P/L (curve configuration ratio) and a lower deformation energy (W), which could lead to stone milled flour producing a lower bread loaf volume and quality compared to roller milled flour. The water absorption also influenced the pasting properties of the flour, as the stone milled flour samples had a lower peak viscosity (Vp) and final viscosity (Vf) than the roller milled flour samples, as well as a shorter peak time. The PCA biplot indicated that the stone milled flour samples were strongest associated with starch damage, P/L, water absorption, ash content, protein content and gluten content, amongst others. Alternatively, the roller milled flour samples were associated with the highest L* (lightness), median particle size, pasting properties and the alveograph L (extensibility).

Future work could address commercial stone milled products and combination milling (i.e. a combination of stone and roller milling). It is likely that commercial stone mills make use of the combination milling method to be able call a wheat flour ‘stone milled’ without compromising on the quality and functionality of the product associated with a higher bran content.

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CHAPTER 4

Evaluation of four commercial white wheat flours with reference to South African wheat flour regulations

Abstract

Previous work indicated that producing a white stone milled wheat flour by first milling the wheat with a commercial stone mill and then sieving the whole wheat flour through a 212 µm laboratory sieve resulted in a flour with a ash content too high to be referred to as a white bread wheat flour according to South African wheat flour regulations. Despite this, white stone milled wheat flour is commercially available from South African points of retail such as supermarkets and retailers. The aim of this study was to evaluate three commercial white stone milled wheat flours and one commercial roller milled wheat flour according to South African regulations that are applicable to wheat flour, as well as other attributes not specified in the regulations. Five batches of each flour sample were collected from points of retail in the Western Cape region, South Africa. The moisture content, ash content, bran content, crude protein content, CIELab colour and the presence of iron were determined. The protein content of the four flour samples was high enough for bread making, however the bran content of the stone milled samples was too high to be classified as 'white bread wheat flour'. The ash and moisture contents were within the regulation limits for all the samples. Not one of the stone milled flour samples indicated a presence of iron, nor was fortification indicated according to regulations on the packaging of these products. The packaging of the roller milled flour samples indicated fortification, however one sample did not have an iron presence. In conclusion, stone milled flour samples have a lower adherence rate to the wheat flour regulations than the roller milled flour samples. Further work regarding the commercial stone milling process is required.

4.1 Introduction

Wheat flour is produced from wheat using predominantly roller or stone milling methods (Doblado-Maldonado *et al*., 2012). The roller milling process produces a white wheat flour by separating the bran and germ from the endosperm before reducing the particle size of the endosperm (Delcour & Hoseney, 2010). Stone milled flour is produced by milling a single wheat stream between two stones, one fixed and one revolving (Gélinas *et al*., 2004). Stone milled flour is often perceived as an artisanal whole wheat product produced on a small-scale (Ross & Kongraksawech, 2018). Consumers believe it to be more nutritional than conventional roller milled flour, resulting in a marketing advantage associated with using the terms 'stoneground' or 'stone milled' flour (Di Silvestro *et al*., 2014; Guerrini *et al*., 2019). Previous studies have indicated that to produce a refined white wheat flour, stone milled whole wheat flour is passed through sieves or plansifters to remove any bran and middlings (Cappelli *et al.*, 2020; Palpacelli *et al*., 2007).

Earlier work showed that white stone milled wheat flour, which was initially a whole wheat flour that had been sieved through a 212 µm laboratory sieve, had a very low flour yield (*ca*. 26-28%) compared to the laboratory roller milled flour (*ca*. 65%), bringing into question if the stone milling process would be economically viable (Chapter 3, section 3.3.2). This contrasted with a recent study by Cappelli *et al.* (2020) that produced a relatively high flour yield (*ca*. 71-78%) of a white stone milled flour with a low ash content (0.48%), which was interesting as the sieve used was 180 µm. The ash content of the stone milled flour (Chapter 3, section 3.3.4) was too high (1.31%) to be classified as a 'white bread wheat flour'. The high ash content, along with the dark brown bran specks, indicated that stone milled flours had a high bran content, mainly due to the stone milling process shattering the whole wheat kernel and distributing the bran throughout the flour.

Stone milled flours are sold in supermarkets and retail points across South Africa, and are used in various products such as breads, rusks, tortillas and biscuits. South African wheat products, which comprise various classes of wheat flour, are regulated to ensure the quality of the final product is fit for consumption (Department of Agriculture, Forestry and Fisheries, 2017). The various wheat flour classes, such as 'cake', 'all-purpose, 'white bread', 'brown bread' and 'whole-wheat' (amongst others), have different stipulations regarding their ash content and wheat bran, germ or semolina contents.

Mandatory fortification of wheat flour is a sustainable and inexpensive method of alleviating micronutrient deficiencies (Allen *et al*., 2006; Department of Health, 2016). Wheat flour is fortified using a fortification mix containing various vitamins and minerals that have been identified as essential for maintaining human health and development yet are deficient in the population (Allen *et al*., 2006). Fortification mix is added to a flour using either gravity or pneumatic systems (Johnson & Wesley, 2010). As specified in the fortification regulations, fortification mixes should consist of the following micronutrients: vitamin A (retinol), vitamin B1 (thiamine), vitamin B2 (riboflavin), vitamin B3 (niacinamide), vitamin B6 (pyridoxine), vitamin B9 (folic acid), vitamin B12 (cyanocobalamin), iron and zinc (Department of Health, 2016).

The aim of this study was to evaluate four commercially available white wheat flours with reference to South African wheat flour regulations. The adherence of the roller milled flour and three stone milled flours to the regulations was determined, as well as comparing the flour samples with each other.

4.2 Material and methods

4.2.1. Wheat samples

White wheat flour samples were anonymously sourced from nine points of retail. The points of retail were in Stellenbosch, Somerset West and Cape Town, Western Cape, South Africa. Flour samples (5 batches of each sample) produced by four wheat flour milling companies were obtained comprising 3 stone milled samples and 1 roller milled sample. The flour samples shall henceforth be referred to as stone mill 1 (SM1), stone mill 2 (SM2), stone mill 3 (SM3) and roller mill (RM). The packaging of some of the flour milling companies did not have batch numbers, thus expiry dates were used to distinguish between batches. Each flour sample was removed from its paper bag packaging, thoroughly mixed and repackaged in resealable plastic bags. The bags were subsequently stored in clean, plastic containers sealed with lids at ambient temperature until further analyses. All analyses were completed within 1 month from date of purchase.

4.2.2. Moisture content

Moisture content analysis of the flour samples was performed in duplicate according to AACC 44-15.02 Air-Oven method (AACC International, 1999c), as described in Chapter 3, section 3.2.5.

4.2.3. Ash content

The AACC method 08-02.01 (ash-rapid (magnesium acetate) method) as described in Chapter 3, section 3.2.5 was used to determine the ash content of the flour samples in duplicate (AACC International, 1999a).

4.2.4. Bran content

The bran content of the flour samples was determined in duplicate according to the method described in the South African wheat product regulations (Department of Agriculture, Forestry and Fisheries, 2017; Appendix 1). Flour samples (200 ± 0.1 g) were sieved through a 200 mm diameter sieve with a 212 µm wire mesh fitted with a receiver container and lid (LABOTEC Test Sieve; Clear Edge Filtration SA Pty (Ltd), South Africa). Nylon cubes (15 mm) were used for efficient sieving and the samples were sieved for 5 min using a horizontal, circular laboratory Sieve Shaker (Scientific Manufacturing cc., Cape Town). Any particles that remained on the bottom of the sieve were brushed into the receiver container. The bran content was determined as the mass of the flour (A) that did pass through the sieve, expressed as a percentage of the total flour.

4.2.5. Presence of iron

AACC Method 40-40.01 was used to qualitatively determine the iron present in the flour samples (AACC International, 1999b). Prepared thiocyanate reagent (*ca*. 1 mL) was placed on a flour sample and allowed to react for 10 min. Using a drip pipet, *ca*. 1 mL of 3% hydrogen peroxide was methodically placed in contact with the same area and allowed to react for another 10 min. Intense red spots developed where iron particles were present.

4.2.6. Crude protein content

The crude protein content of the flour samples was determined in duplicate according to AACC method 46-30.01 (Dumas combustion method; AACC International, 1999d) using the Gerhardt Dumatherm DT N40+ (Gerhardt Analytical Systems, Königswinter, Germany). The nitrogen conversion factor of 5.7 was used to calculate the protein content expressed on a 12% moisture basis.

4.2.7. Colour

The Konica Minolta Spectrophotometer CM-5 (Chiyoda City, Tokyo, Japan) was used to conduct CIELab colour analysis to determine the L* (lightness), a* (red-green value) and b* (blue-yellow value) of the flour samples in duplicate, as described in Chapter 3, section 3.2.5.

4.2.8. Statistical analyses

STATISTICA version 13.6 (StatSoft Inc., Tulsa, Ok, USA) was used to perform statistical analysis. Mixed model analysis of variance (ANOVA) using lme4 package in R was performed to compare the mean differences between the flour samples. The data was presented as mean ± standard deviation. The different post hoc analyses was done using the Fisher least significant (LSD) test. A 95% confidence interval, or 5% significance level ($P \leq 0.0.5$), was used to identify significant results.

4.3 Results and discussion

4.3.1. Rationale of flour sampling and analysis

All white wheat flour samples were anonymously obtained from the points of retail to ensure that the samples were representative of the products purchased by the public. The millers did not have prior knowledge of the samples being sourced. Methods of analysis were according to those specified in *Regulations relating to the grading, packing and marking of wheat products intended for sale in the Republic of South Africa* and shall henceforth be referred to as the wheat flour regulations (Department of Agriculture, Forestry and Fisheries, 2017; Appendix 1). This regulation also stipulates packing and marking requirements, as well as quality standards for wheat products. Fortification requirements are documented in the *Regulations relating to the fortification of certain foodstuffs* and shall henceforth be referred to as the fortification regulations (Department of Health, 2016; Appendix 2).

4.3.2. White wheat flour sample classification

According to wheat flour regulations, the wheat product class must be specified on the packaging (Department of Agriculture, Forestry and Fisheries, 2017). The class of the flour samples SM1, SM2 and RM have been indicated as 'white bread wheat flour', which is a subclass of 'white wheat flour'. However, SM3 has been marked as 'coarse white' and 'coarse white wheat flour' on respectively the front and side panels. This is not a defined wheat product class in the regulations.

The South African wheat flour regulations do not define or differentiate between flours obtained from either the roller or stone milling process. Due to the differences in the final product, mainly because of the grinding severity and milling process, one could expect provisions to be made for stone milling. This could include classes with specific reference to stone milled flour, either as white or brown wheat flour. The stone milled flour samples clearly indicated that they were 'stoneground' or 'stone ground' flour. The terms 'unbleached', 'natural' and 'traditional' were also used in combination with 'stoneground' and 'stone ground' to describe the stone milled flours. This may be due to the marketing advantage associated with using the term 'stoneground' (Di Silvestro *et al.*, 2014), as well as the belief that stone milled flour is healthier and more nutritious than roller milled flour (Guerrini *et al.*, 2019).

The packaging of all the flour samples indicated the mass of the contents, the name and/or trademark and a physical address of the respective milling companies.

To indicate the required fortification of white wheat flour in South Africa, the packaging must be labelled with the official fortification logo and the claim 'Fortified for better health' (Department of Health, 2016). Neither of these were displayed on the packaging of SM1, SM2 or SM3; nor did the ingredients list and nutritional table indicate the presence of vitamins, minerals and trace elements. The packaging of RM fulfilled the above-mentioned fortification requirements, including the official logo in monochrome.

4.3.3. Moisture, ash and bran content

Excessive moisture may detrimentally affect the shelf life and microbial safety of flour. The moisture content of SM1, SM2, SM3 and RM (Figure 4.1a) were within the stipulated 14 $\pm$ 0.2% for white wheat flours (Department of Agriculture, Forestry and Fisheries, 2017).

According to wheat flour regulations, the ash content of white bread wheat flour must be between 0.6 ± 0.05% and 1.0 ± 0.05% (Department of Agriculture, Forestry and Fisheries, 2017). Wheat flour regulations differ from country to country, as can be seen by the difference in the ash content requirements. For example, Austrian regulations stipulate that the ash content of white flour should be 0.33-0.58%, the United Kingdom 0.48-0.63% and the French government requires white bread wheat flour to have an ash content of 0.5-0.75% (Zanirato, 2013). All four flour samples in this study, except for one batch (batch 3) of SM1, were within the stipulated South African ash content limits (Figure 4.1b). The ash content indicates the amount of inorganic material in a flour and is higher in the outer layers of the wheat kernel than the inner layers (Hinton, 1959; Kim & Flores, 1999). Ash content is used as an indicator of the bran contamination in white flours, and thus the milling efficiency. Bran reduces the quality of bread by modifying the crumb texture, causing a darker crumb colour and a decreased loaf volume (Delcour & Hoseney, 2010; Zhang & Moore, 1999). Hence, no separated wheat bran, wheat germ or wheat semolina must be present in white bread wheat flour (Department of Agriculture, Forestry and Fisheries, 2017). The bran contents of the stone milled samples (SM1, SM2 and SM3) were significantly ($P \leq 0.05$) higher than that of the roller mill sample (RM) (Table 4.1). This may be because the stone milling process crushes the entire wheat kernel into a flour, thus distributing finer bran particles throughout the flour (Gélinas *et al*., 2004). Stone milling results in the flour particles being either very large or very fine, which means that sieving could be ineffective in removing the smaller bran particles to produce a white flour (Chapter 3; Gélinas *et al*., 2004). During the roller milling process, the wheat kernel is sheared open using very precisely gapped rollers, enabling the endosperm to be separated from the larger bran particles using a sieving system. The endosperm, which has a lower ash content than the bran, is then reduced in size to a flour (Delcour & Hoseney, 2010; Hinton, 1959).

Commercial stone millers often employ a combination of stone and roller milling to optimise milling efficiency and minimise bran contamination, but still maintain the marketing advantage associated with stone milling (Doblado-Maldonado *et al.*, 2012; Posner & Hibbs, 2011). This is done by cracking the wheat kernel open using a stone mill and then reducing the size of the flour using a roller mill. It is also possible that commercial stone mills that employ combination milling practices first use a roller mill to separate the bran and endosperm before reducing the size of the endosperm with a stone mill. The bran content of SM3 (39.29 ± 3.38%) was nearly ten times ($P \leq 0.05$) that of SM1 (4.20 ± 0.99%) and SM2 (4.34 ± 1.88%) (Table 4.1). This may be because SM1 and SM2 make use of combination milling methods, whereas SM3 might be 100% stone milled. It may also be due to the differences in the production processes of the stone milling companies (first stone, then roller mill or vice versa) or the mill itself (different brands and models).

The effect of stone size, dressing and abrasiveness as well as the settings of the mills (such as feed rate, rotational speed and distance between stones) may also influence the flour (Cappelli *et al*., 2020; Gélinas *et al*., 2004; Ross & Kongraksawech, 2018). RM was the only flour sample that had a bran content (0.08 ± 0.02%) that was within the regulation requirements for white bread wheat flour, as the regulation allows for a maximum tolerance level of 0.5% (Figure 4.1c). The high bran content of SM3 (which is too high to be classified as a white bread wheat flour) and the low ash content indicate that this product is misclassified as 'coarse white wheat flour', which is not a wheat product class stipulated in the regulations (Department of Agriculture, Forestry and Fisheries, 2017). This product could be classified as a 'high bran wheat flour', as the bran was more than 15% and the ash content below 1%.

It was expected that the higher bran content of the stone mills would be reflected in the ash content results, yet this was not the case (Hinton, 1959; Kim & Flores, 1999). The ash content of SM3, which had a significantly higher ($P \leq 0.05$) bran content than the other samples, was not higher nor did it significantly differ from the other flour samples ($P=0.62$). This anomaly may have to do with the bran content analysis method that is stipulated in the wheat flour regulations (Department of Agriculture, Forestry and Fisheries, 2017). This method is based on the bran and germ having a larger particle size (>212 µm) than the endosperm (<212 µm) as is typical with roller milling. This allows the bran and endosperm to be separated when the flour is passed through a 212 µm sieve. The stone milled samples may have larger (>212 µm) flour particles which are not bran particles which remain on the sieve, which could also result in the correlation between ash and bran content being incorrect in this case. As the stone milled flours are often contaminated with bran, an alternative bran quantification method, such as the bran speck count test, may be more effective (Kim & Flores, 1999).

The addition of fortified micronutrients, such as iron and zinc, may have caused the ash content of the RM sample to be higher than it would have been if it had not been fortified (Akhtar *et al.*, 2008). As the packaging of SM1, SM2 and SM3 did not contain fortification claims or micronutrients listed in the nutritional table and ingredients list, nor did they indicated a presence of iron during analysis (Table 4.2), it is likely that if these samples had been fortified, it may have resulted in an even higher ash content.

4.3.4. Protein content

Despite the protein content being one of the factors determining the grade of bread wheat in South Africa, wheat flour protein is not regulated. Protein content influences the final product's functionality, as it affects the water absorption and loaf volume (Carson & Edwards, 2009; Gabriel *et al*., 2017). Wheat flour with a lower protein content of 7-11% is usually intended for biscuits and cake production, whereas wheat flour intended for bread production has a minimum protein content of 12% (Carson & Edwards, 2009).

The protein content of wheat is influenced by several factors such as the farming conditions (fertilisation and weather), as well as the cultivar. The four flour samples in this study have a high protein content of *ca*. 13-14%, making them suitable for breadmaking (Figure 4.1d).

Table 4.1 The mean moisture, ash, protein and bran content, as well as the L*, a* and b* values of the four flour samples assessed with the mixed model ANOVA

Flour sample	**Moisture content (%)** (P≤0.05)	**Ash content (%)** (P=0.62)	**Protein content (%)** (P=0.10)	**Bran content (%)** (P≤0.05)	**Colour**		
					L* (P≤0.05)	**a*** (P≤0.05)	**b*** (P≤0.05)
SM1	13.70 ± 0.53^{a}	0.67 ± 011^{a}	13.81 ± 0.44^{a}	4.20 ± 0.99^{b}	91.12 ± 0.37^{c}	0.91 ± 0.10^{b}	10.59 ± 0.52^{b}
SM2	13.31 ± 0.16^{b}	0.70 ± 0.02^{a}	13.12 ± 0.52^{b}	4.34 ± 1.88^{b}	91.49 ± 0.41^{b}	0.97 ± 0.13^{b}	10.17 ± 0.33^{c}
SM3	13.08 ± 0.20^{b}	0.69 ± 0.05^{a}	13.70 ± 0.99^{a}	39.29 ± 3.38^{a}	88.44 ± 0.53^{d}	1.44 ± 0.14^{a}	12.02 ± 0.45^{a}
RM	13.77 ± 0.17^{a}	0.71 ± 0.13^{a}	13.58 ± 0.48^{ab}	0.08 ± 0.02^{c}	93.25 ± 0.12^{a}	0.60 ± 0.03^{c}	10.04 ± 0.25^{c}

Values are mean ± standard deviation of 5 batches in duplicate
Mean values with different superscripts in a column differ significantly (P≤0.05)

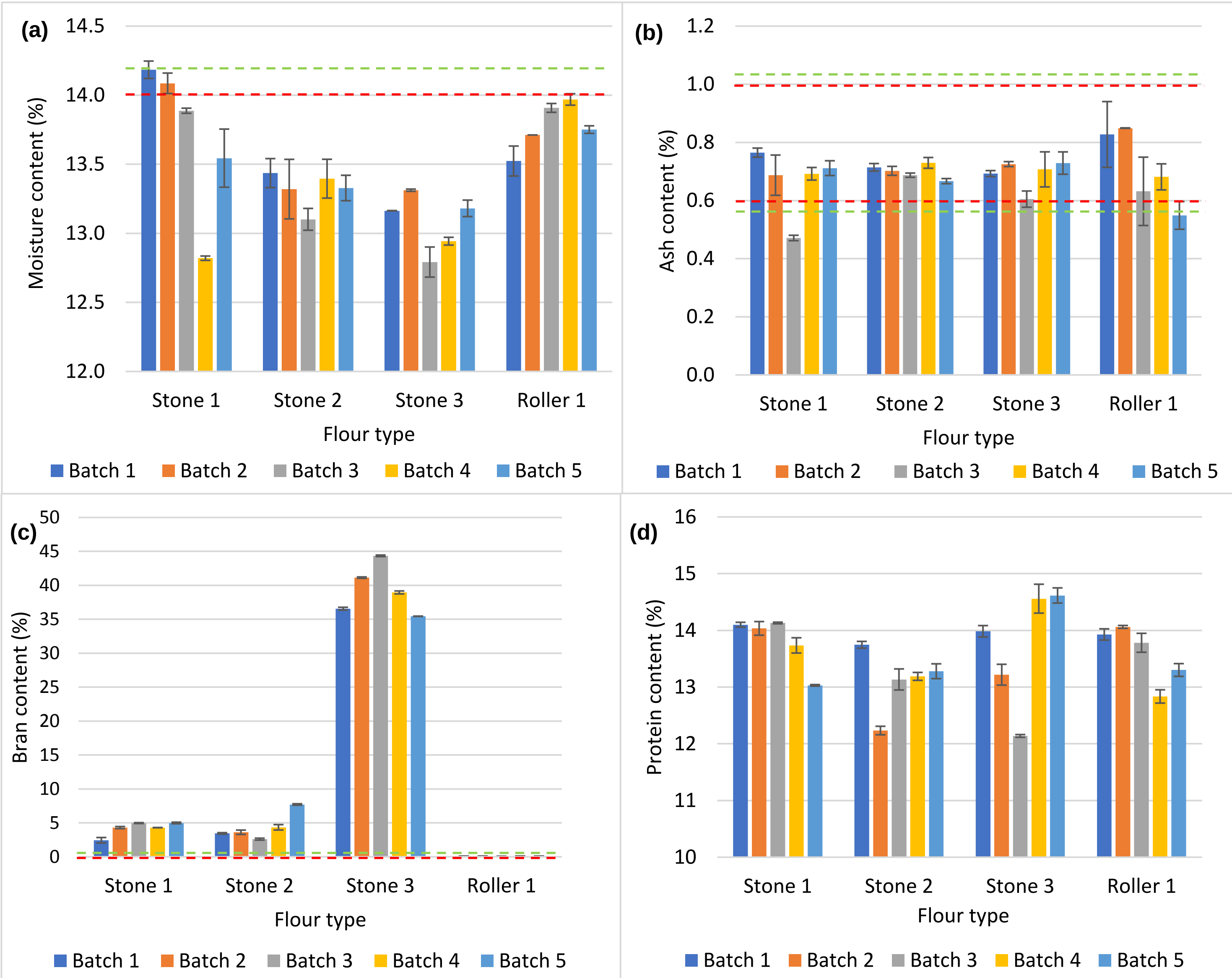

Figure 4.1 The (a) moisture, (b) ash, (c) bran and (d) protein content of four commercially available wheat flour samples, with the dashed lines indicating the limits (red) and tolerance levels (green) according to South African wheat flour regulations.

4.3.5. Presence of iron

All white wheat flours, including white bread wheat flour, should be fortified with the specified micronutrients (Department of Agriculture, Forestry and Fisheries, 2017; Department of Health, 2016). In terms of wheat flour, micronutrients such as iron are added to wheat products using a fortification mix.

A rapid method that gives an indication if a flour is fortified is by determining if iron is present. This method is only qualitative and does not indicate all the micronutrients that should be used in fortification. However, it does give an indication if the fortification mix is present and distributed throughout the flour (AACC International, 1999b). According to the fortification regulations, fortification mix should contain ferric sodium ethylenediaminetetraacetate (NaFeEDTA). This is because NaFeEDTA enhances iron absorption by preventing phytates from binding to it, as well as inhibiting lipid oxidation (Hurrel, 1997). During the storage of flour, ferrous iron (Fe^{2+}) may oxidise to ferric iron (Fe^{3+}) and is influenced by environmental factors such as storage temperature and humidity (Hemery *et al*., 2018). However, the analysis method used in this study oxidises any ferrous iron (using hydrogen peroxide) to ferric iron and will therefore account for all the iron present in the sample (AACC International, 1999b).

Despite the fact that RM adhered to the labelling stipulations regarding fortification, only 80% of the batches tested positive for the presence of iron (Table 4.2). Possible reasons for this may be drawn back to the production of the flour. A fortification mix might not have been added to the flour batch due to human error; or it was added and was not mixed properly, thus not distributing the fortification mix evenly amongst product units (Johnson & Wesley, 2010). The fortification mix could also have been of a poorer quality and contained an insufficient amount of iron and other micronutrients (Yusufali *et al*., 2012). However, this is less likely as fortification mixes must comply to standards set out in the fortification regulations, which entails accredited laboratory (South African Bureau of Standards Pharmaceutical Chemistry Laboratory) analysis and audits (Department of Health, 2016; Yusufali *et al*., 2012).

The stone milled flour samples (SM1, SM2 and SM3) did not indicate an iron presence (Table 4.2). This may be because stone milled flour was traditionally established as a niche whole wheat product used by artisanal bakers. This whole wheat flour was produced on a small scale using only stone milling and was not fortified. In recent years, the demand for local stone milled flour has increased, and a range of flours is produced on a much larger scale, including white wheat flour. It is thus likely that stone millers continued with the practice of not fortifying flour. It is also likely that fortification is not enforced since stone milled flour is not stipulated as such in the fortification regulations. The initial capital costs of implementing a fortification system might also be too high for small-scale mills, such as the stone mills (Johnson & Wesley, 2010).

Table 4.2 Qualitative analysis of presence of iron (%) of the five batches of four flour samples

Flour sample	Iron present (%)	Iron not present (%)
SM1	0	100
SM2	0	100
SM3	0	100
RM	80	20

RM was the only flour sample that adhered to all the regulations regarding the limits for the moisture, ash and bran content (Table 4.3). It was also the only flour sample that indicated an iron presence in 80% of the batches, compared to SM1-3 (0%). The stone mills also had a 0% adherence rate for the bran content, however all the batches (100%) of SM2 and SM3 were within the limits for the ash content, with SM1 following at 80%.

Table 4.3 Adherence of four different flour sample's batches (%) to South African wheat flour regulation limits for moisture content, ash content, bran content and iron presence

Flour sample	Moisture content (%)	Ash content (%)	Bran content (%)	Presence of iron (%)
SM1	100	80	0	0
SM2	100	100	0	0
SM3	100	100	0	0
RM	100	100	100	80

4.3.6. Flour colour

The colour of wheat flour is not stipulated in South African regulations, yet it plays an important role in consumer acceptability, especially as a white flour is expected to be white. The CIELab L* value indicates the whiteness or brightness of a flour (Figure 4.3a), the a* the redness (Figure 4.3b) and the b* the yellowness (Figure 4.3c). The closer the L* is to 100, the whiter the flour. RM produced the whitest flour that had a significantly higher ($P \leq 0.05$) L* value than the stone mills (SM1-3). It was also the least red (lowest a*) and yellow (lowest b*) compared to the stone milled flour samples (Table 4.1).

SM1 and SM2 were similar in colour, however SM3 was noticeably non-uniform with darker coloured bran specks (Figure 4.3). SM3, which was also called a 'coarse white wheat flour', had the highest bran content and this is reflected in the lowest L* and highest a* values. A more uniform colour could be produced by reducing the bran content and particle size (Zhang & Moore, 1999). The particle size of stone milled flour could be reduced by tightening the stones (decreasing the distance between the two stones) (Gélinas *et al*., 2004). To reduce the bran content of the flour, the use of combination milling may be effective in first separating the bran before reducing the endosperm, thus ensuring a whiter flour.

Figure 4.2 Image illustrating the colour difference of the four flour samples, where the first row is SM1, followed by SM2, SM3 and RM (batches 1-5 is indicated from left to right).

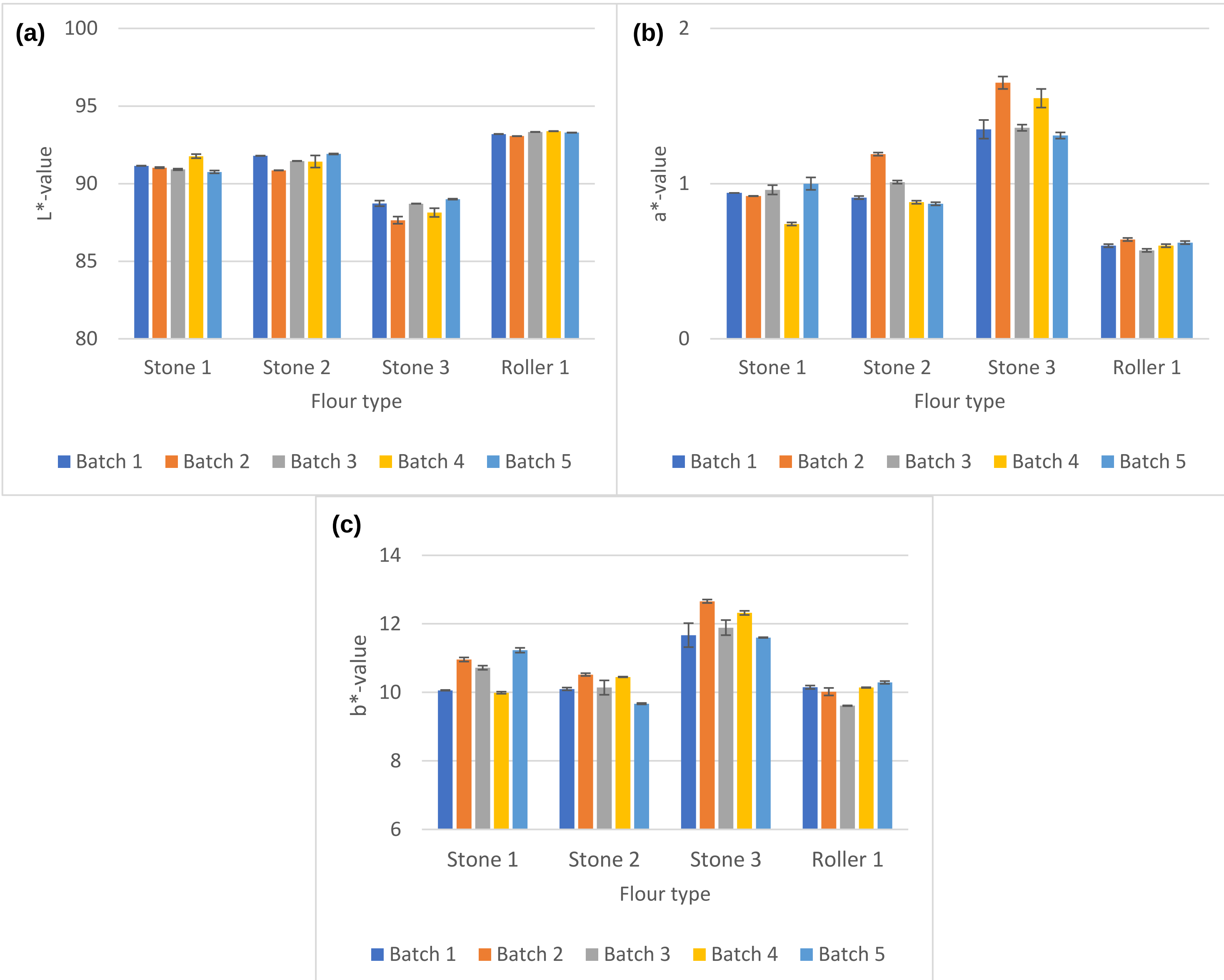

Figure 4.3 CIELab colour results of four commercially available wheat flours: (a) L* value, (b) a* value and (c) b* value.

4.4 Conclusions

The three stone and one roller milled flour samples all showed various levels of adherence to South African wheat flour regulations. Flour samples SM1, SM2 and RM were marked as 'white bread wheat flour' on the packaging. SM3, which was marked as 'coarse white wheat flour', was the only sample that did not classify as a known class according to South African wheat flour regulations. This flour sample could be classified as a 'high bran wheat flour'. The protein contents of the four flour samples were suitable for bread production, and the moisture contents were within the stipulated 14%. All the stone milled flour samples (except one SM1 batch) were within the ash content limits for a white bread wheat flour classification. However, the bran content for the stone milled flour samples was too high for the classification of white bread wheat flour. The packaging of the stone milled flour samples was not marked according to fortification regulations and did not indicate a presence of iron, which implies that no fortification took place. The roller milled sample (RM) was the only sample that adhered to the ash and bran content regulations, and four of the five batches indicated a presence of iron. RM was also the whitest sample, whereas SM3 was the darkest due to bran particles. Overall, the roller milled flour samples had a higher rate of adherence to the regulations than stone milled flour samples.

The differences between the fundamental milling process of stone and roller milling should be considered as this affected the quality of the flour samples. The results of this study present a foundation for future studies, as more research regarding the commercial stone milling process and products is required. This study was limited to regional stone milling companies. It is recommended that a larger range of stone and roller milled flour products be investigated in the future with samples collected over a longer time period. A quantitative nutritional analysis (especially the amounts of riboflavin, nicotinamide and retinol as these are specified in fortification regulations) would complement the qualitative iron analysis test in this study and should be considered in the future to further verify fortification of the flour samples. It is also recommended that a more efficient bran content analysis method be employed than the bran sieve method indicated in the wheat flour regulations, as it distinguishes bran from endosperm flour particles based exclusively on the particle size. This is problematic as stone milling reduces the entire wheat kernel to a flour and does not have a bran and endosperm separation step as with roller milling, thus resulting in bran being distributed throughout the flour. Provision could be made for stone milled flour in South African wheat flour regulations as the differences in the stone and roller milling processes affect the flour quality. Provision in the regulations may provide more clarity for stone millers regarding product requirements and the production process. This study established that there is variance amongst stone milled flours, indicating differences in the production process of stone milled flours. Therefore, comprehensive further research on this topic is required before such an amendment can be made as very little is known about the commercial stone milling process.

4.5 References

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CHAPTER 5
General discussion and conclusion

Most commercial wheat flour mills produce flour using roller milling methods, however local and small-scale stone mills are experiencing a resurgence in popularity (Ross & Kongraksawech, 2018). This may be because stone milled flour has a distinct marketing advantage associated with the terms 'stone milled' or 'stoneground' (Di Silvestro *et al*., 2014). Stone milled flour is believed to be more nutritious than roller milled flour, as it is mostly produced from smaller quantities of local, organic or ancient wheats (Guerrini *et al*., 2019; Kihlberg *et al*., 2004; Ross & Kongraksawech, 2018). Previous studies indicated that micronutrients and major elements of wheat samples were retained when they were stone milled to a whole wheat flour, whereas the roller milled refined flour showed a reduction in heavy metals and these elements (Albergamo *et al*., 2018; Cubadda *et al*., 2003; Cubadda *et al*., 2009). This may be because these studies compared whole wheat stone milled flour with a refined roller milled flour. The stone milling process in these studies did not remove the external wheat layer where these nutrients are found, whereas the roller milled flour did in order to produce a white flour.

The production processes of roller and stone milled white flour differ. Roller milling separates the bran and germ from the endosperm using corrugated and smooth metal rolls and oscillating sieves. The endosperm is then reduced in size to produce a refined white flour (Delcour & Hoseney, 2010; Posner & Hibbs, 2011). The production of white stone milled flour differs as the entire wheat kernel is ground between two stones to produce a whole wheat flour (Cappelli *et al*., 2020; Palpacelli *et al*., 2007). The whole wheat flour is then passed through a sieve system. Previous studies mostly focused on whole wheat stone milled flour and presented contrasting results. Whole wheat stone milled flour was said to have higher levels of starch damage and water absorption than roller milled flour (Gèlinas *et al*., 2004; Prabhasankar & Rao, 2001; Ross & Kongraksawech, 2018), which was in contrast with Kihlberg *et al.* (2004). It was also said that stone milled flour had a very large particle size (Palpacelli *et al*., 2007), very small particle size (Gélinas *et al*., 2004; Ross & Kongraksawech, 2018) or a gradual increase in particle size (Kihlberg *et al*., 2004).

Limited work has been published on white stone milled flour properties. Cappelli *et al.* (2020) analysed the effect of the stone rotational speed and wheat tempering of a weak, ancient wheat on the rheology, particle size distribution, flour yield and the mill's productivity and specific energy consumption. The only other study on white stone milled wheat flour was concerning the mycotoxin content of white stone milled flour, which was lower than that of roller milled flour (Palpacelli *et al*., 2007). The aim of the first research chapter (Chapter 3) was to address the knowledge gap regarding the physicochemical, functional and structural properties of white stone milled flour, and how it compared to roller milled flour. The stone milled flour samples had a very low white flour yield (26.19-28.39%) compared to the roller milled samples (64.56-65.03%). This may be due to the efficiency of small-scale laboratory practices used, or it may be due to blockages in the stone mill due to the grain being softened during tempering. A previous study on whole wheat stone milled flour indicated that wheat should not be tempered before milling as this would lead to the wheat sticking to the furrows or grooves of the stone (Gélinas *et al*., 2004). In contrast, Cappelli *et al*. (2020) optimised white wheat flour yield by tempering the wheat before stone milling. By tempering wheat to 13 and 15% moisture content, an optimal white wheat flour yield of 73.3-77.8% was achieved. Despite the fact that the wheat was tempered to a similar moisture content in this study (*ca*. 15%) and the sieve openings were larger (212 µm vs 180 µm) than that used by Cappelli *et al.* (2020) the low white flour yield of the stone milled flour brought into question the economic viability therof.

It was established that the ash content of sifted stone milled flour was too high to be classified as a white flour according to South African wheat flour regulations (Department of Agriculture, Forestry and Fisheries, 2017). The stone milled flour also had a significantly darker colour ($P \leq 0.05$) than the roller milled flour, with bran specks clearly visible. This was due to the sieving of the whole wheat stone milled flour being ineffective in the separation of the bran. The stone milling process reduced the entire wheat kernel to a flour and did not separate the bran and endosperm before size reduction as with roller milling, resulting in bran being distributed throughout the flour. A similar occurrence of bran contamination was established in a previous study (Gèlinas *et al*., 2004), which contrasted with studies that obtained white stone milled flour production with an acceptable ash content (Cappelli *et al*., 2020; Palpacelli *et al.*, 2007). The current study established that stone milled flour had a significantly higher starch damage ($P \leq 0.05$) than roller milled flour due to the grinding severity of the millstones. The stone milled flour also had a significantly higher falling number ($P \leq 0.05$) than the roller milled flour. The median particle size of the stone milled flour was significantly smaller ($P \leq 0.05$) than the roller milled flour. The finer particles of the stone milled flour, along with the increased levels of starch damage and ash content, resulted in a significantly higher water absorption capacity ($P \leq 0.05$) compared to the roller milled flour. This resulted in the gluten being inhibited and forming a stiffer dough. Alveograph and mixograph properties were affected by this, as the stone milled flour was significantly less extensible ($P \leq 0.05$) and had a high curve configuration ratio, with significantly longer ($P \leq 0.05$) optimal mixing times and peak heights than the roller milled flour. The Rapid Visco Analyser (RVA) pasting analysis results indicated that the stone milled flour had significantly lower viscosities (P≤0.05), possibly due to the high starch damage levels and smaller particle size. Scanning electron micrographs (SEM) illustrated the qualitative microstructural differences between stone and roller milling. Stone milled flour was more inhomogeneous than roller milled flour and had large bran particles that were clearly visible. Stone milled flour samples also had frequent damaged starch granules that had a deformed shape or were cracked or split.

The question was raised of how commercially milled white stone milled flour would compare to roller milled flour, as well if stone milled white flour adhered to South African wheat flour (Department of Agriculture, Forestry and Fisheries, 2017) and fortification regulations (Department of Health, 2016). This was addressed in the second research chapter (Chapter 4). White stone milled flour is found on the South African market in supermarkets, retailers and wholesalers. Two of the three stone mills labelled the flour product as 'white bread wheat flour', which is stipulated to have an ash content of 0.6 $\pm$ 0.05% and 1.0 $\pm$ 0.05% and a maximum bran content of 0.05% according to wheat flour regulations (Department of Agriculture, Forestry and Fisheries, 2017). The third stone milled flour sample was labelled as 'coarse white wheat flour', which is not an official wheat flour class in South African regulations. This flour sample could actually be classified as a 'high bran wheat flour' as the bran was more than 15% and the ash content below 1% (Department of Agriculture, Forestry and Fisheries, 2017). The stone milled flour samples had significantly (P≤0.05) higher bran contents than the roller milled flour sample. The bran content of the flour samples, determined according to the wheat flour regulations using a sieving method, did not reflect the ash content (P=0.62) of the stone milled flour samples. This may be because the bran content method distinguishes bran as having a larger particle size (>212 µm) than the rest of the flour (<212 µm). The roller milling method separates the bran and endosperm before the endosperm is reduced in size to a flour, however this is not true for stone milled flour as the entire wheat kernel is reduced in size, resulting in fine bran particles being distributed throughout the flour. This was in contrast to previous work that indicated that the ash content of a wheat kernel decreases from the outer layers to the inner layers (Hinton, 1959). The stone milled flour samples, especially the 'coarse white' flour, were significantly darker (lower L* value) in colour than the roller milled flour, which may be due to the presence of very fine bran particles that were distributed throughout the flour. The other two stone milled samples may be whiter than the 'coarse white' flour sample due to the use of combination milling methods or differences in the milling process.

The presence of iron was used to qualitatively determine if the flour was fortified. None of the stone milled flour samples indicated a presence of iron, nor did the packaging of these samples contain the stipulated fortification logo and claims or the nutritional information that indicate the presence of vitamins and minerals used in fortification mixes. All the packaging of the roller milled batches indicated the fortification stipulation and all the batches (except for one) indicated a presence of iron. The absence of iron in the one roller milled batch may be due to human error (not adding the fortification mix to the flour), insufficient mixing of the flour and the fortification mix or the use of a poor quality fortification mix that did not contain sufficient iron. As the iron presence test is qualitative, future work should further verify the levels of fortification by determining the micronutrient contents in flour samples as the fortification mix added may contain insufficient quantities.

It is possible that some of the commercial stone mill flour samples were produced using a combination of stone and roller milling methods (Chapter 4). Combination milling consists of a stone mill that first cracks the wheat kernel open before a roller reduces it to a flour (Doblado-Maldonado *et al*., 2012) however it is likely that commercial stone millers first use a roller mill and then a stone mill. Future studies on the commercial stone milling process is needed to corroborate if and how the combination milling method is used, as well as if the effect it has on the physicochemical and functional properties of wheat flour.

The knowledge obtained in this study may serve as a foundation for future work on refined stone milled flour. An evaluation of established stone mills and their processes and products may lead to wheat flour regulations being changed or adapted to be more inclusive, as it was indicated in this study that there is variance amongst stone milled flour of different companies. This may include a study of variables such as the tempering, dressings of the millstones, ventilation, heat generation, feed rates and aperture, as well as a study of combination milling. These stone milled flours should be analysed for other physicochemical and functional properties that are not stipulated in wheat flour regulations (such as starch damage, pasting properties, alveographs and mixographs, to name a few). A more extensive nutritional analysis of stone milled flour, including a quantification of fortified vitamins and mineral is needed. The contrasting results obtained in this study regarding the ash and bran content is also something that should be looked at in future studies, including the bran content analysis method stipulated in the wheat flour regulations. All the above-mentioned recommendations would provide a better understanding of stone milled flour and ensures that the miller, baker and consumer is more informed.

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Appendix 1

Regulations relating to the grading, packing and marking of wheat products intended for sale in the Republic of South **(Department of Agriculture, Forestry and Fisheries, 2017)**

To view Appendix 1, follow the link:

http://extwprlegs1.fao.org/docs/pdf/saf167502.pdf

Appendix 2

***Regulations relating to the fortification of certain foodstuffs* (Department of Health, 2016)**

To view Appendix 2, follow the link:

http://www.gpwonline.co.za/Gazettes/Gazettes/39776_3-3_Health.pdf